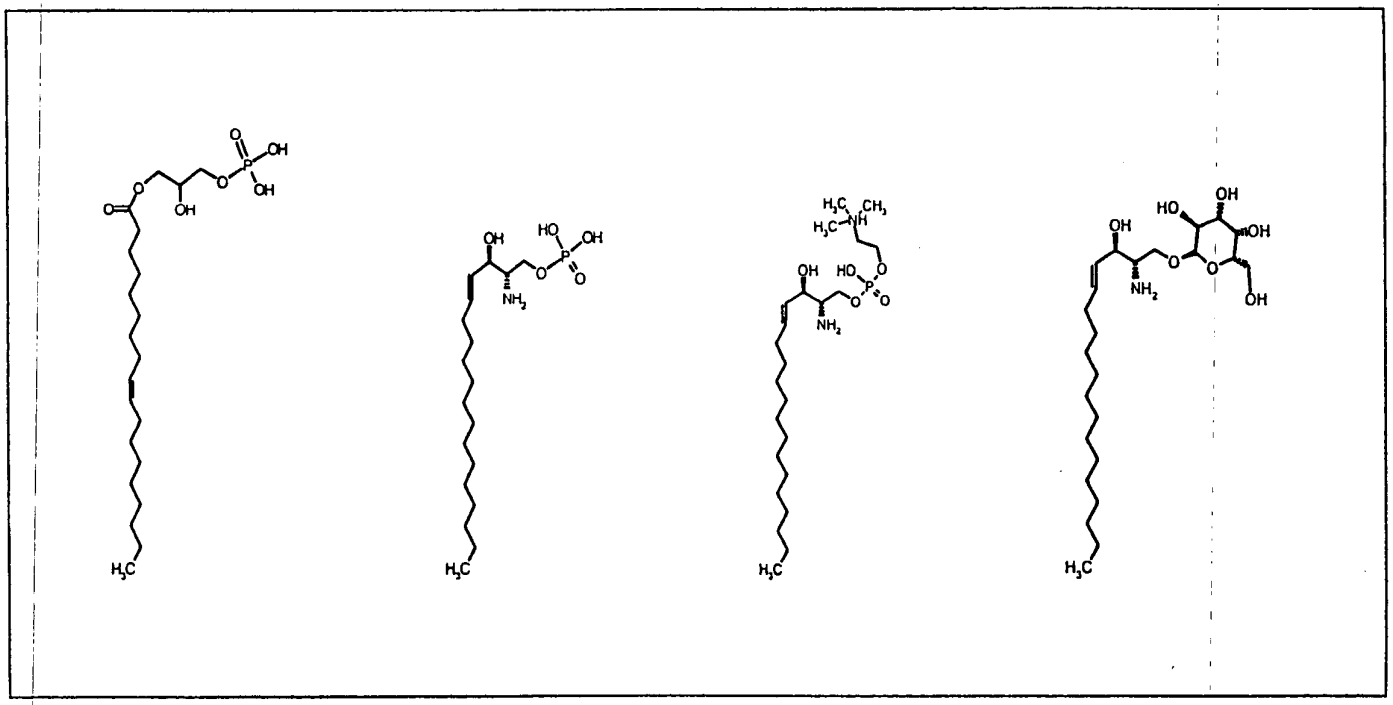
Figure 1A.



LPA **S1P** **SPC** **Psychosine**

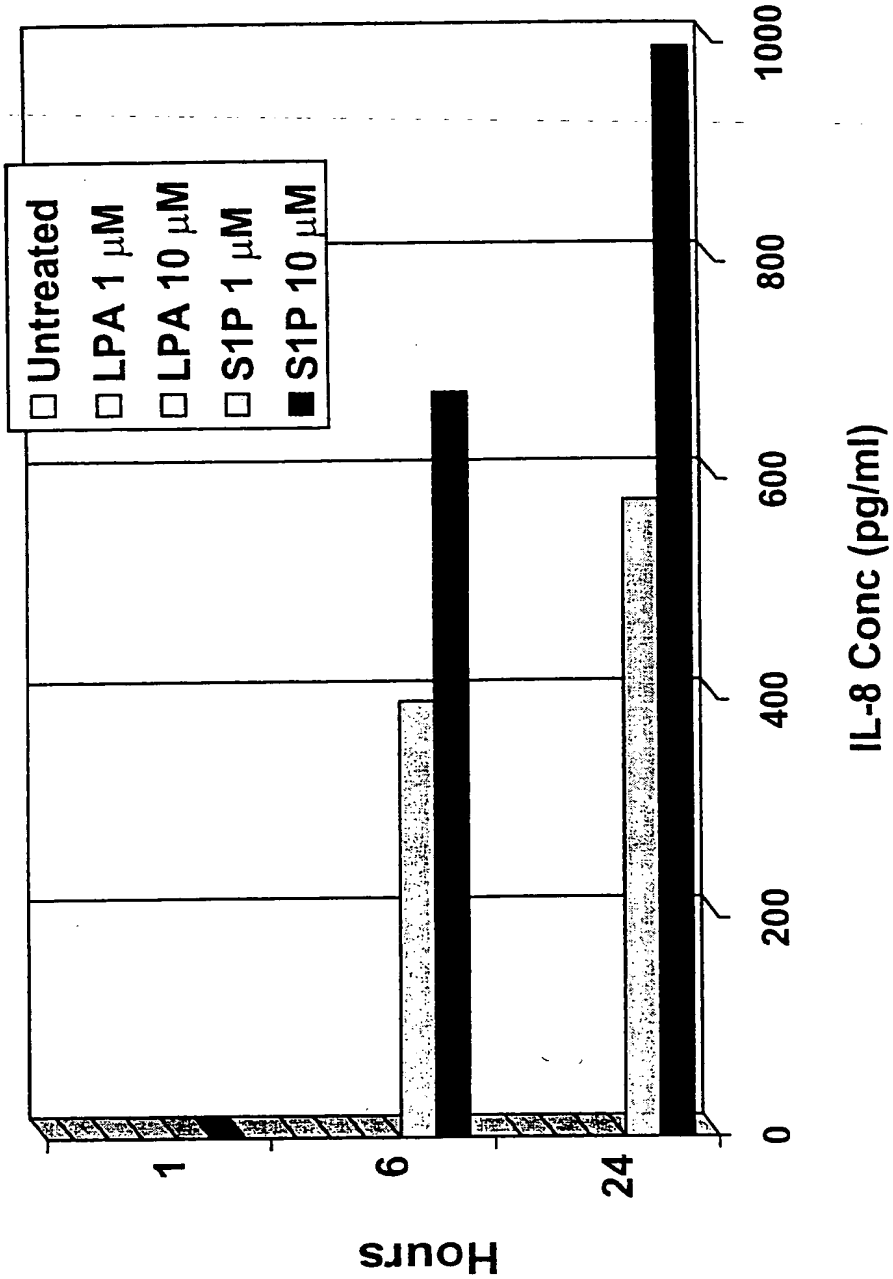

Untreated
LPA 1 μM
LPA 10 μM
S1P 1 μM
S1P 10 μM
0
200
400
600
800
1000
IL-8 Conc (pg/ml)
1
6
24
Hours

Figure 1B.

Figure 2A.

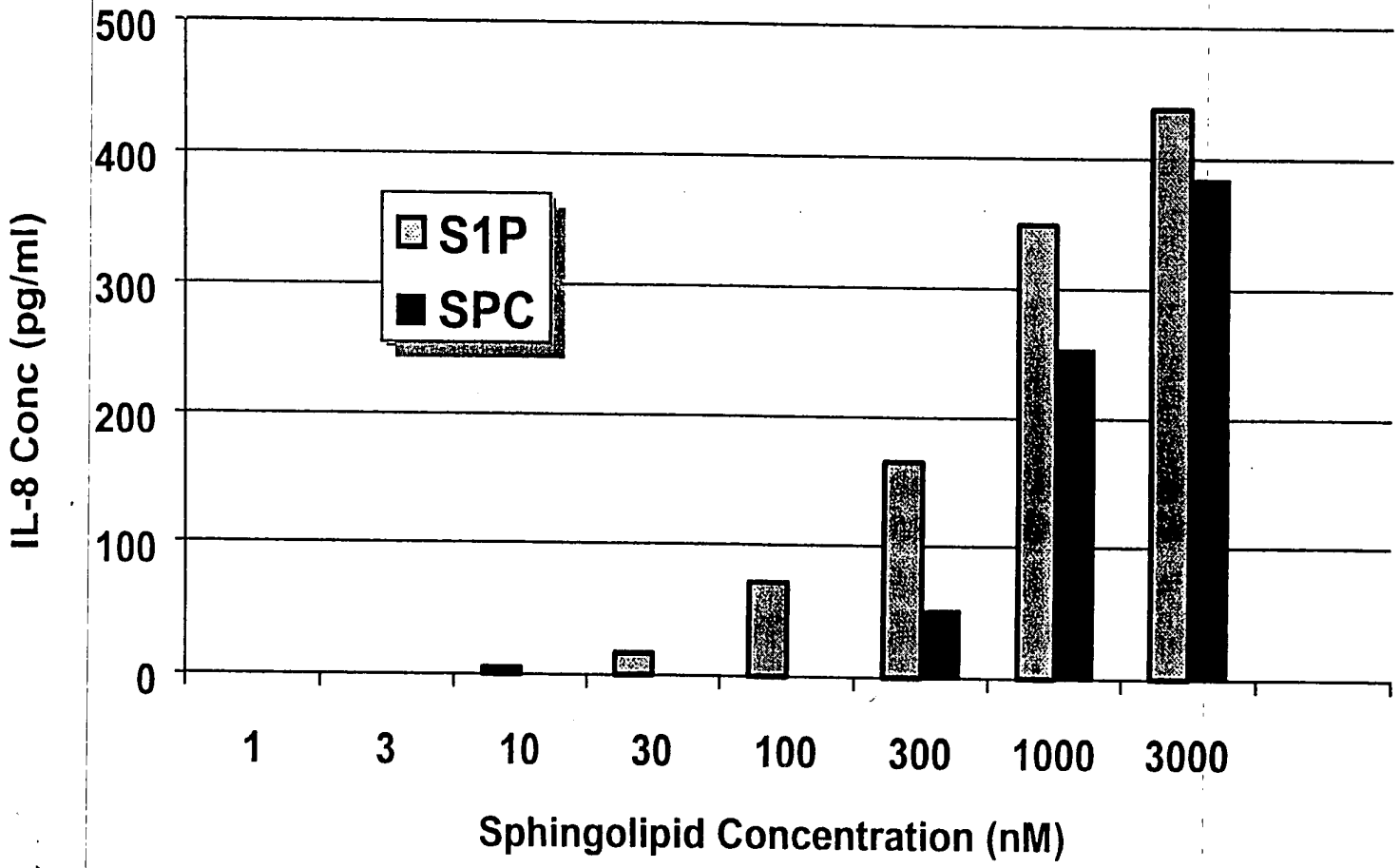

500
400
300
200
100
0
IL-8 Conc (pg/ml)
S1P
SPC
1
3
10
30
100
300
1000
3000
Sphingolipid Concentration (nM)

FIGURE 2B

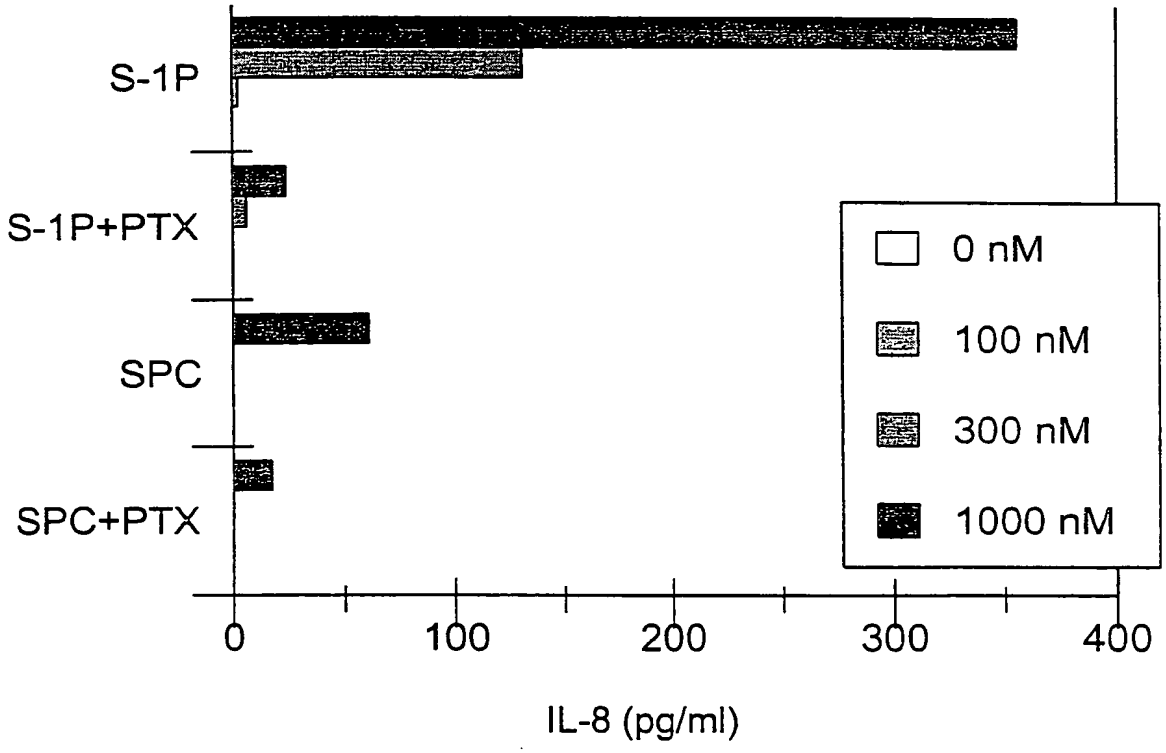

S-1P
S-1P+PTX
SPC
SPC+PTX
0 nM
100 nM
300 nM
1000 nM
0
100
200
300
400
IL-8 (pg/ml)

Figure 3.

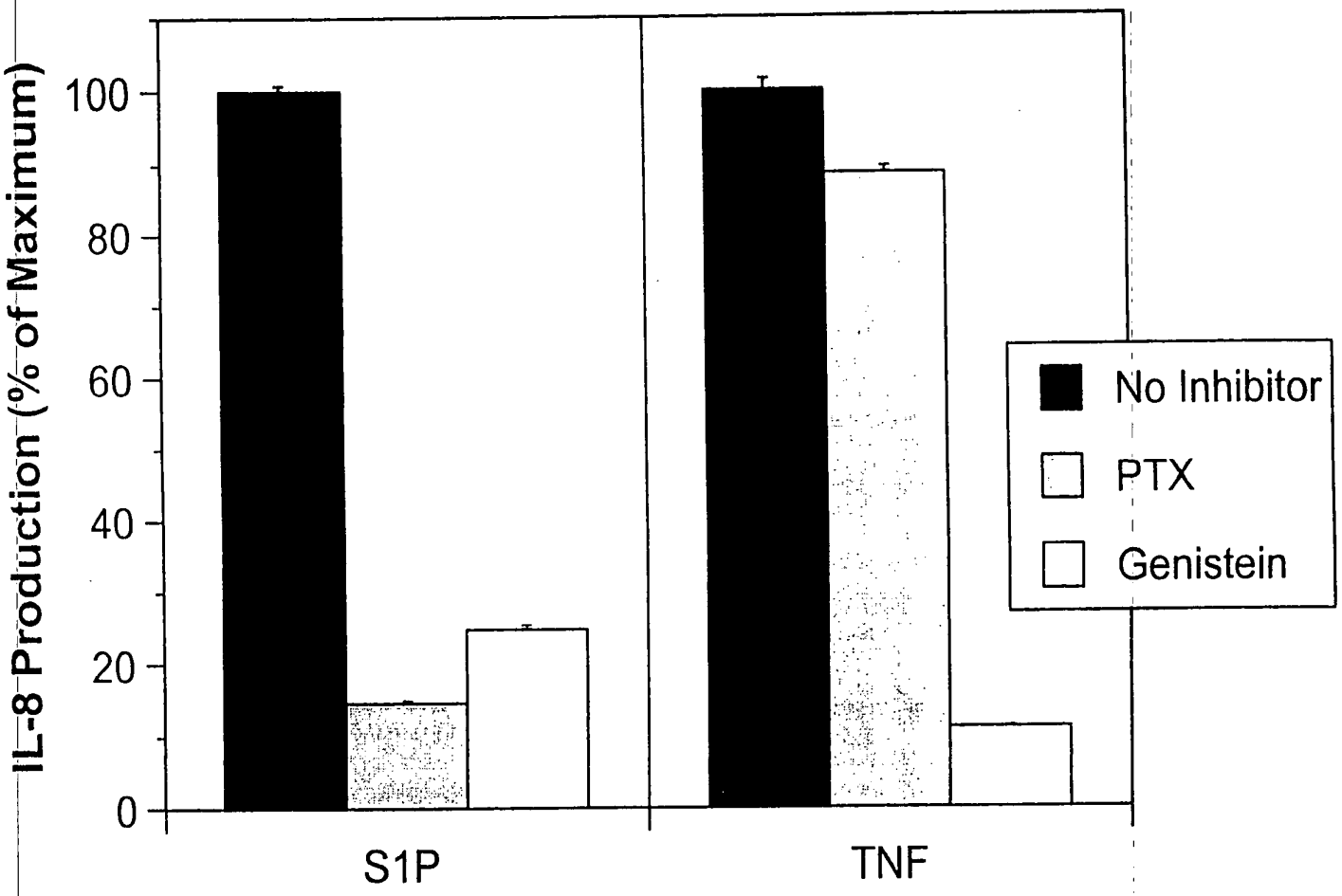

IL-8 Production (% of Maximum)
100
80
60
40
20
0
S1P
TNF
No Inhibitor
PTX
Genistein

FIGURE 4A

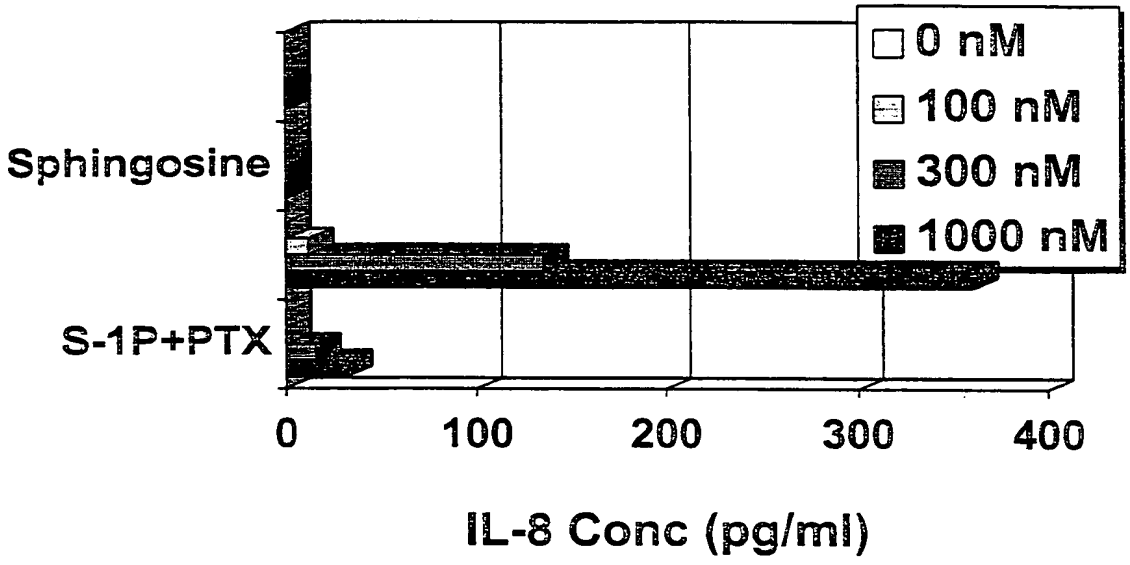

0 nM
100 nM
300 nM
1000 nM
Sphingosine
S-1P+PTX
0
100
200
300
400
IL-8 Conc (pg/ml)

Figure 4B.

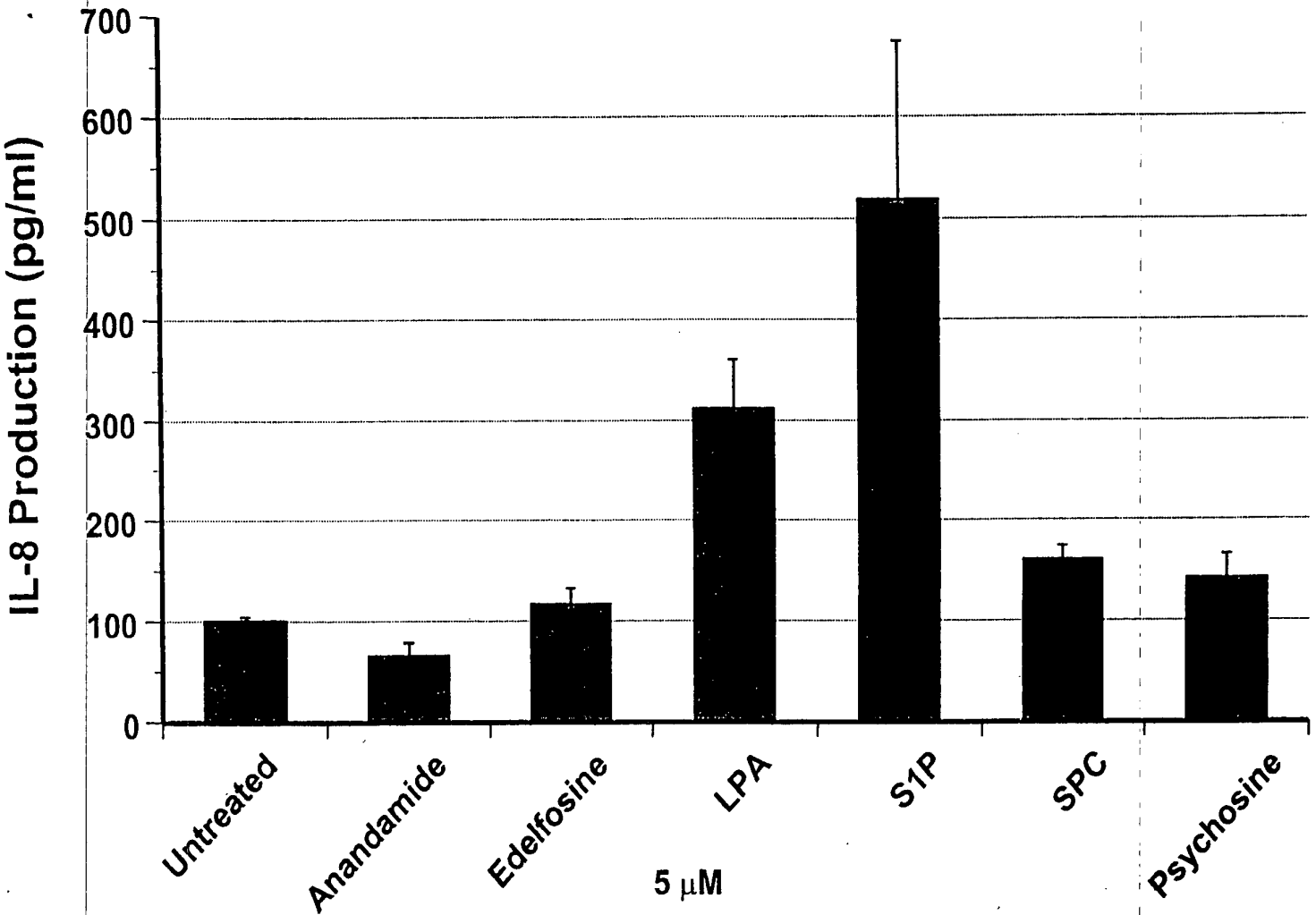

IL-8 Production (pg/ml)
700
600
500
400
300
200
100
0
Untreated
Anandamide
Edelfosine
LPA
S1P
SPC
Psychosine
5 μM

Figure 5.

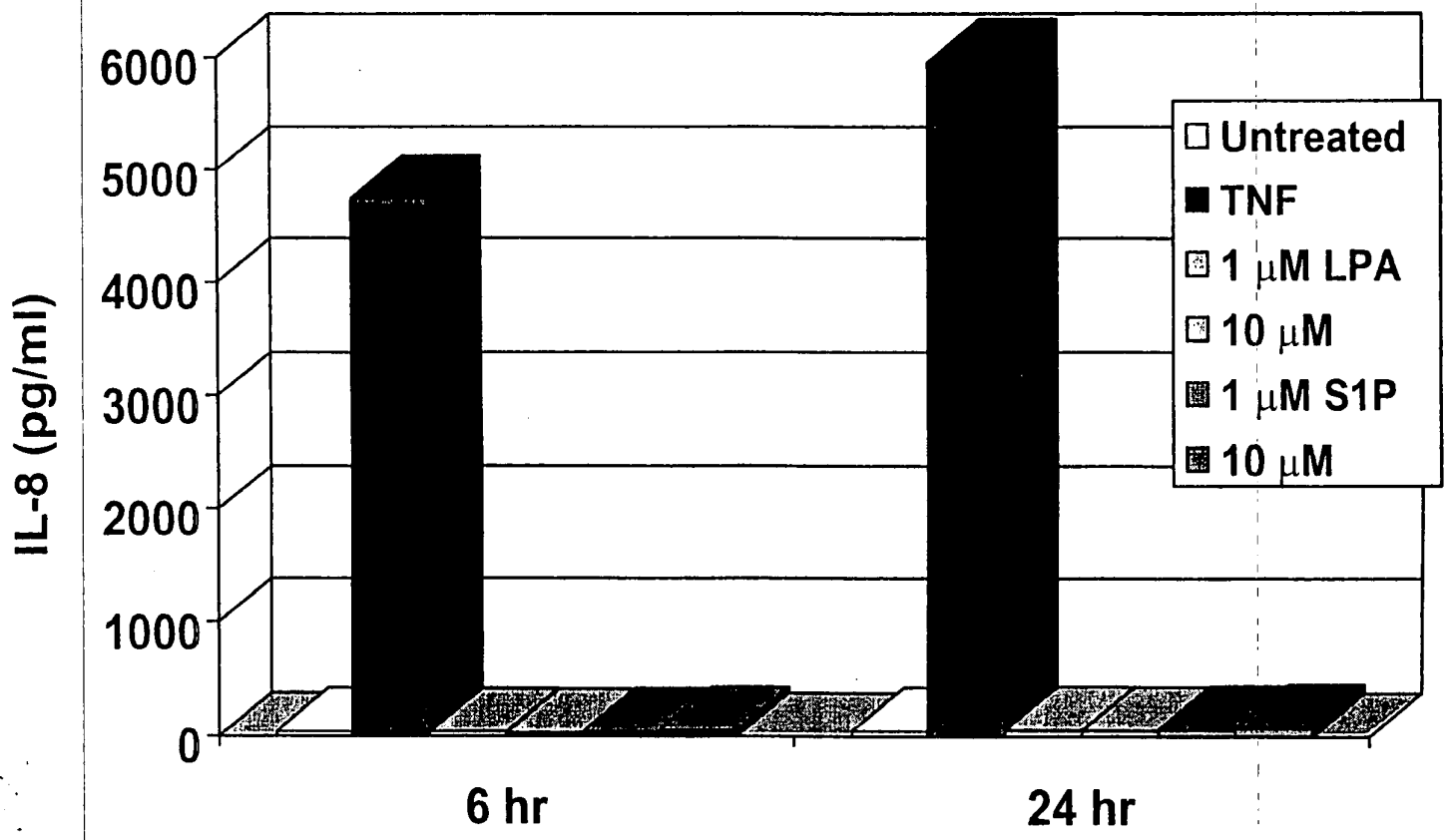
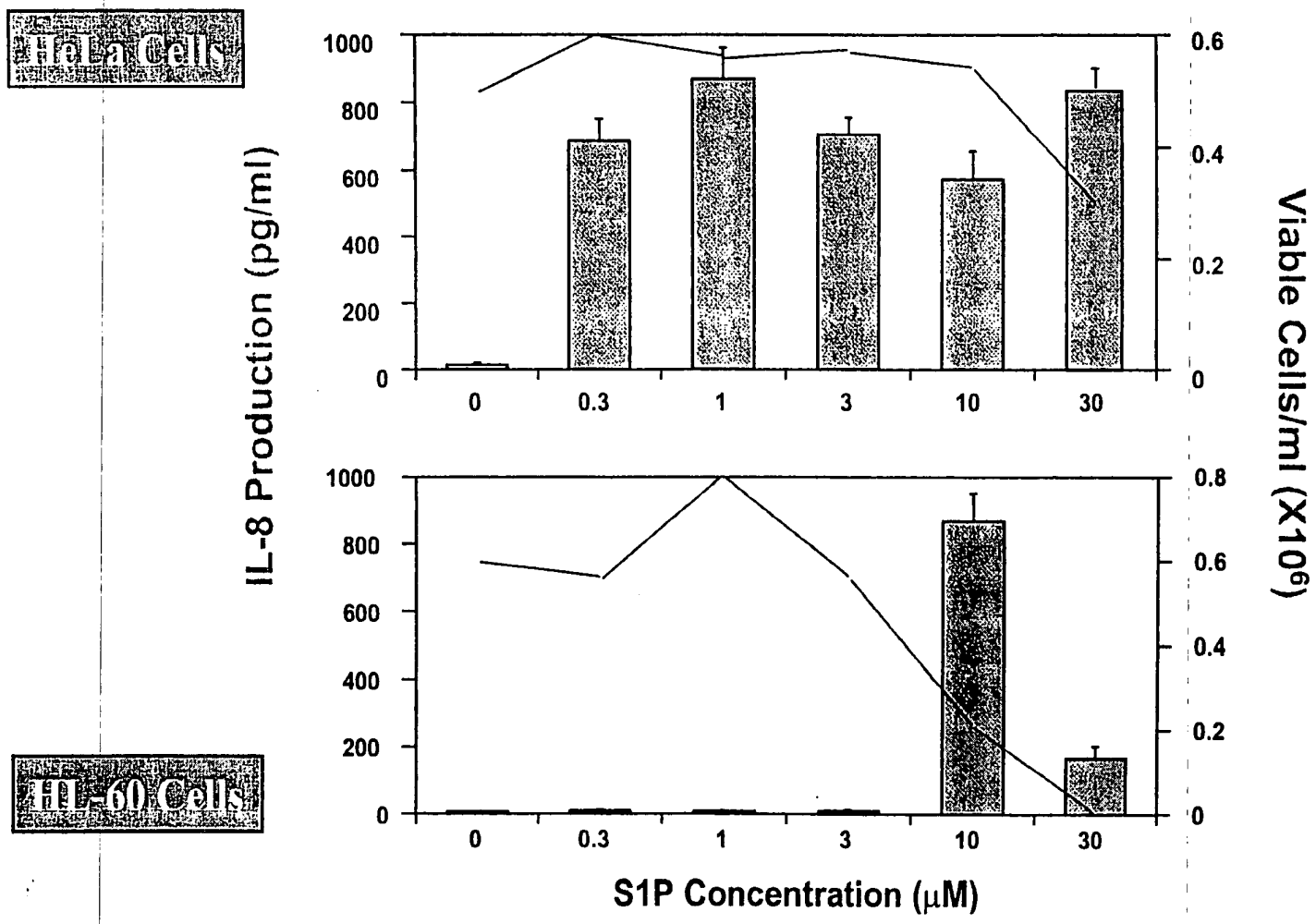

IL-8 (pg/ml)
6000
5000
4000
3000
2000
1000
0
6 hr
24 hr
Untreated
TNF
1 μM LPA
10 μM
1 μM S1P
10 μM

Figure 6.


HeLa Cells
HL-60 Cells
IL-8 Production (pg/ml)
Viable Cells/ml (X10⁶)
1000
800
600
400
200
0
0.6
0.4
0.2
0.8
0
0.3
1
3
10
30
S1P Concentration (μM)

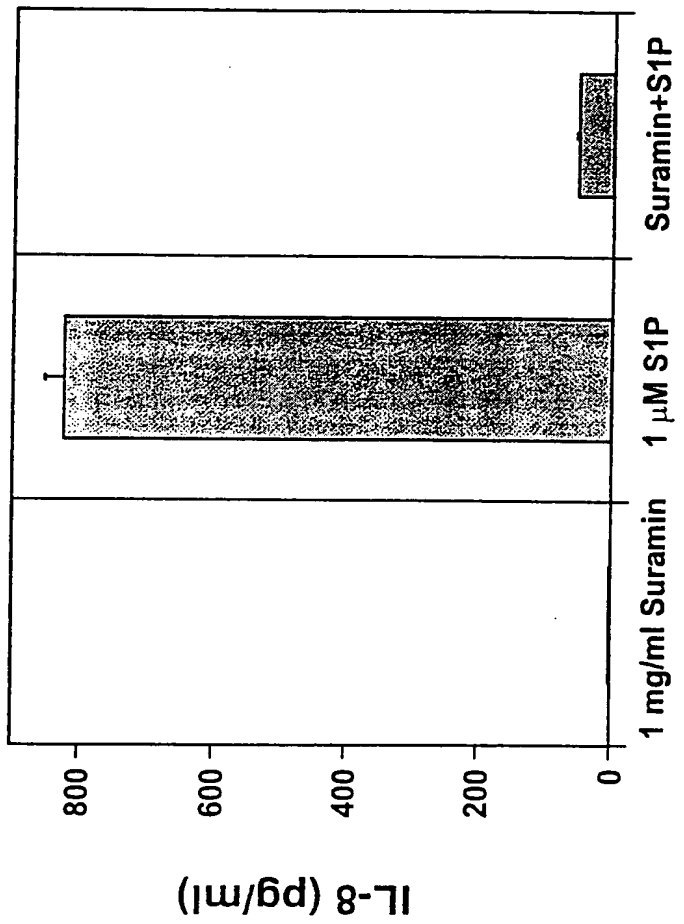


Figure 7.

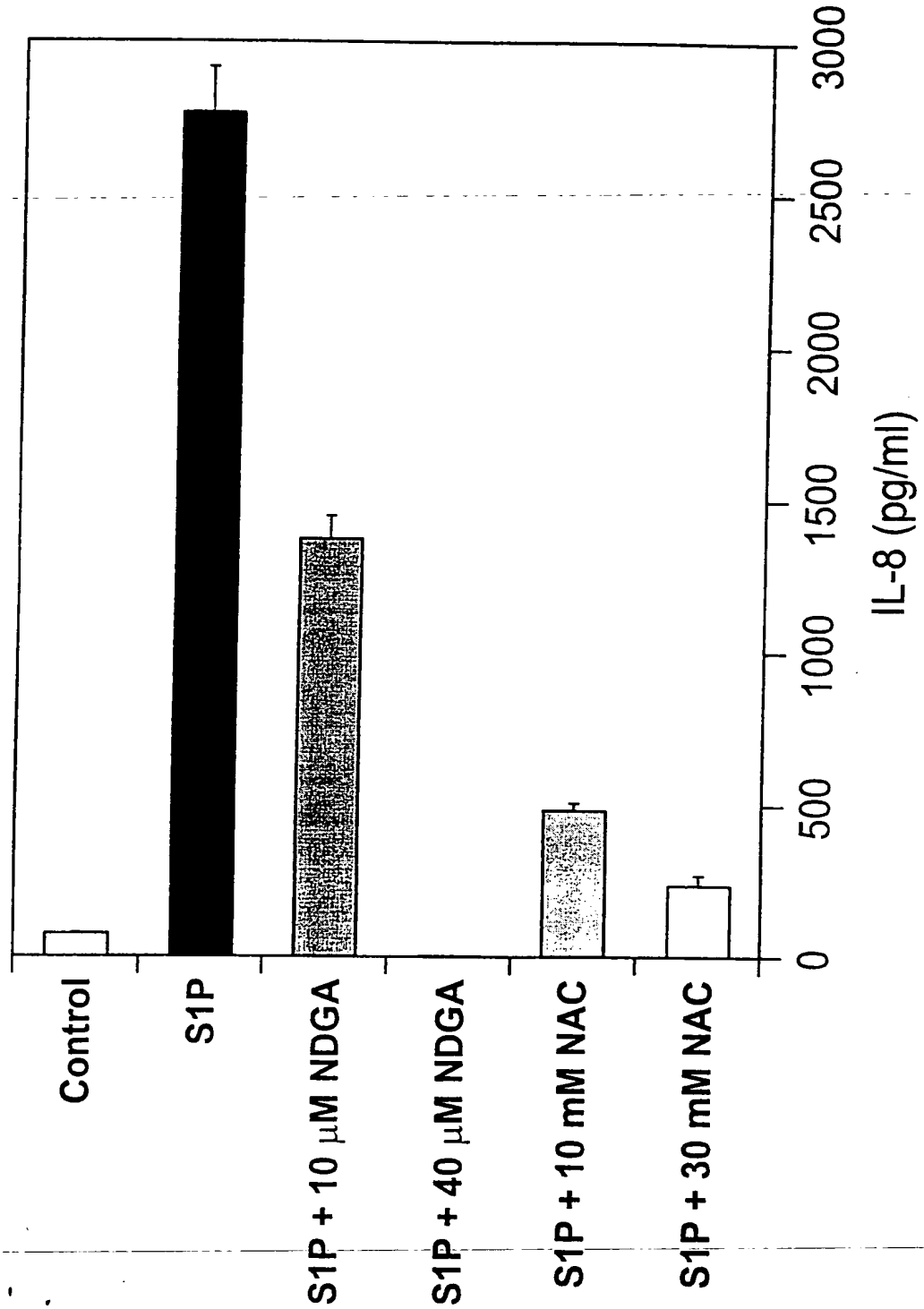

Control
S1P
S1P + 10 μM NDGA
S1P + 40 μM NDGA
S1P + 10 mM NAC
S1P + 30 mM NAC
0
500
1000
1500
2000
2500
3000
IL-8 (pg/ml)

Figure 8.

Figure 9.

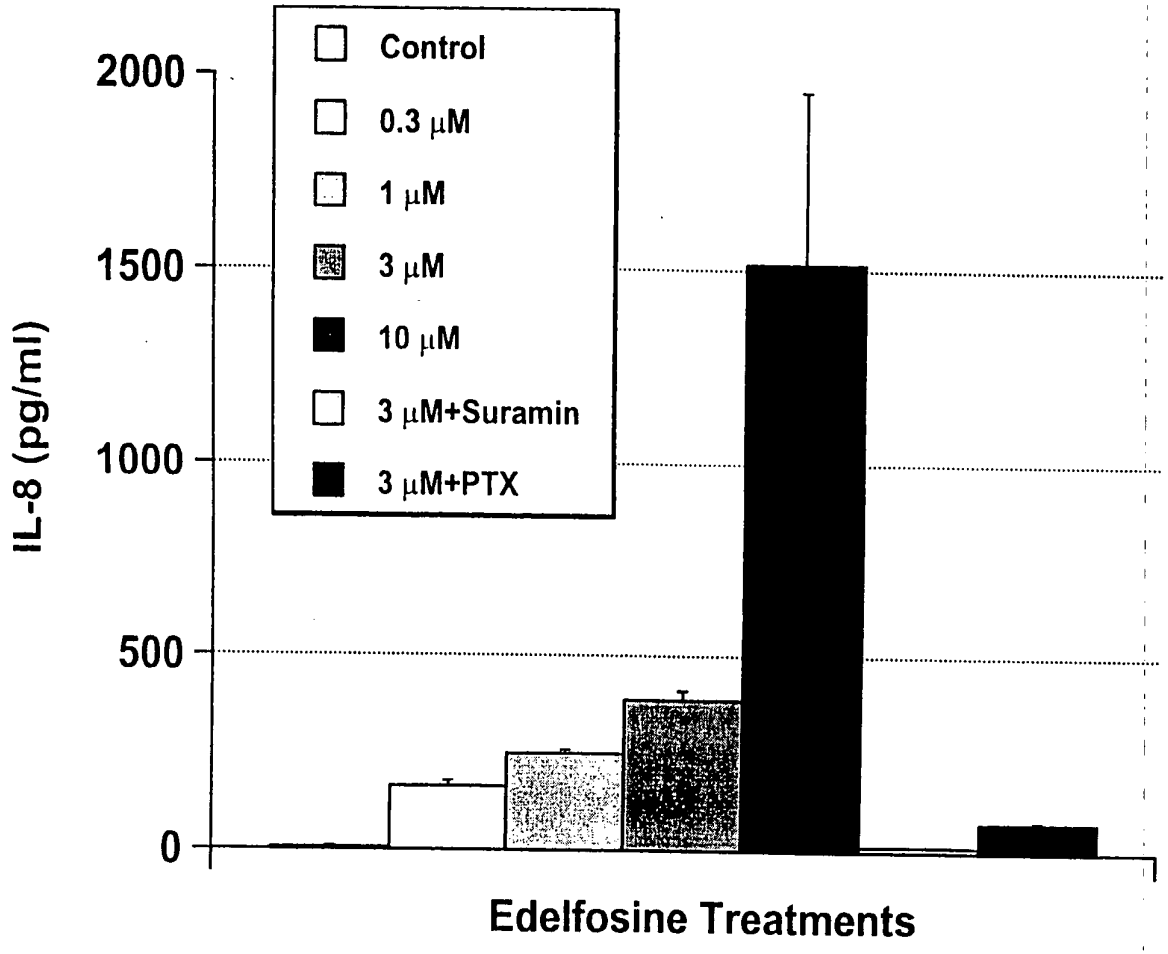

Control
0.3 μM
1 μM
3 μM
10 μM
3 μM+Suramin
3 μM+PTX
2000
1500
1000
500
0
IL-8 (pg/ml)
Edelfosine Treatments

FIGURE 10A

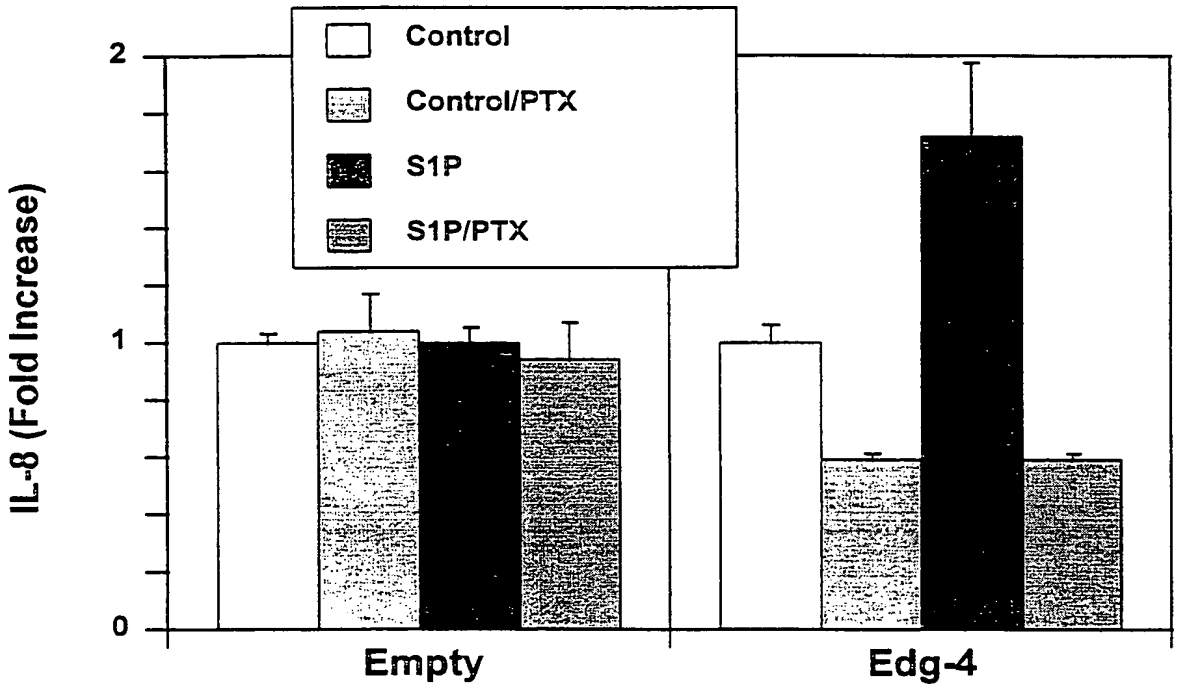
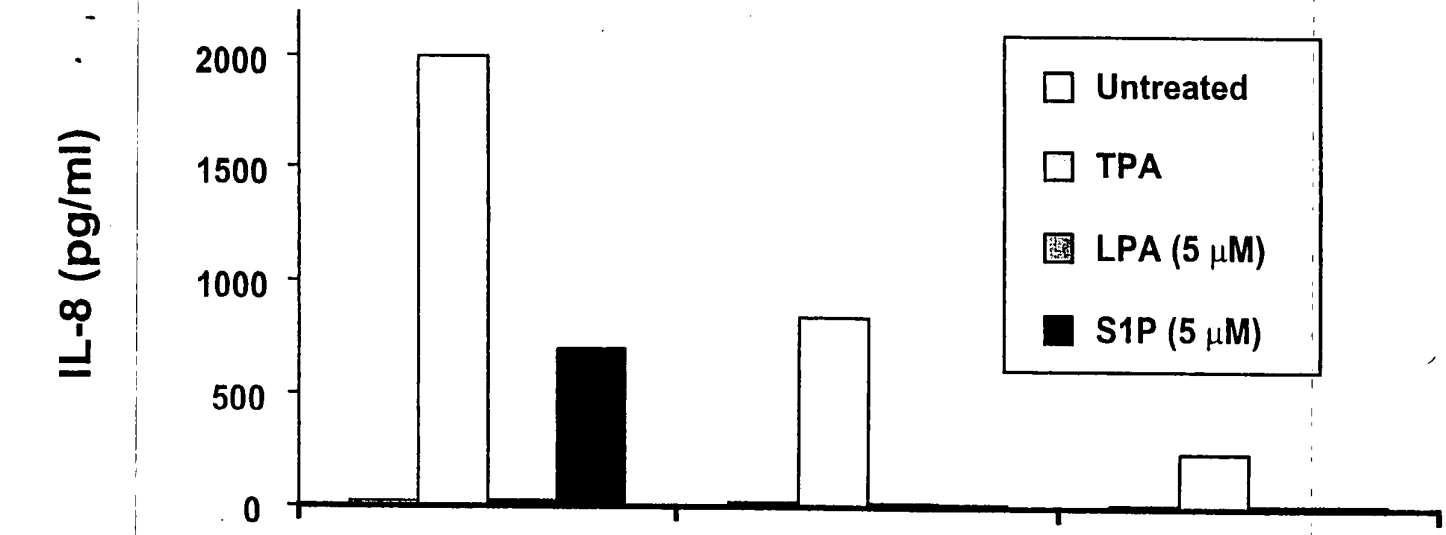

Control
Control/PTX
S1P
S1P/PTX
IL-8 (Fold Increase)
2
1
0
Empty
Edg-4

Figure 10B



Edg RNA	HeLa	COS	293-EBNA
1	+/-	+	-
2	+/-	+	-
3	++	+++	-
4	++	-	-
5	+/-	+	+
6	-	+/-	-
7	-	-	-

Figure 11.

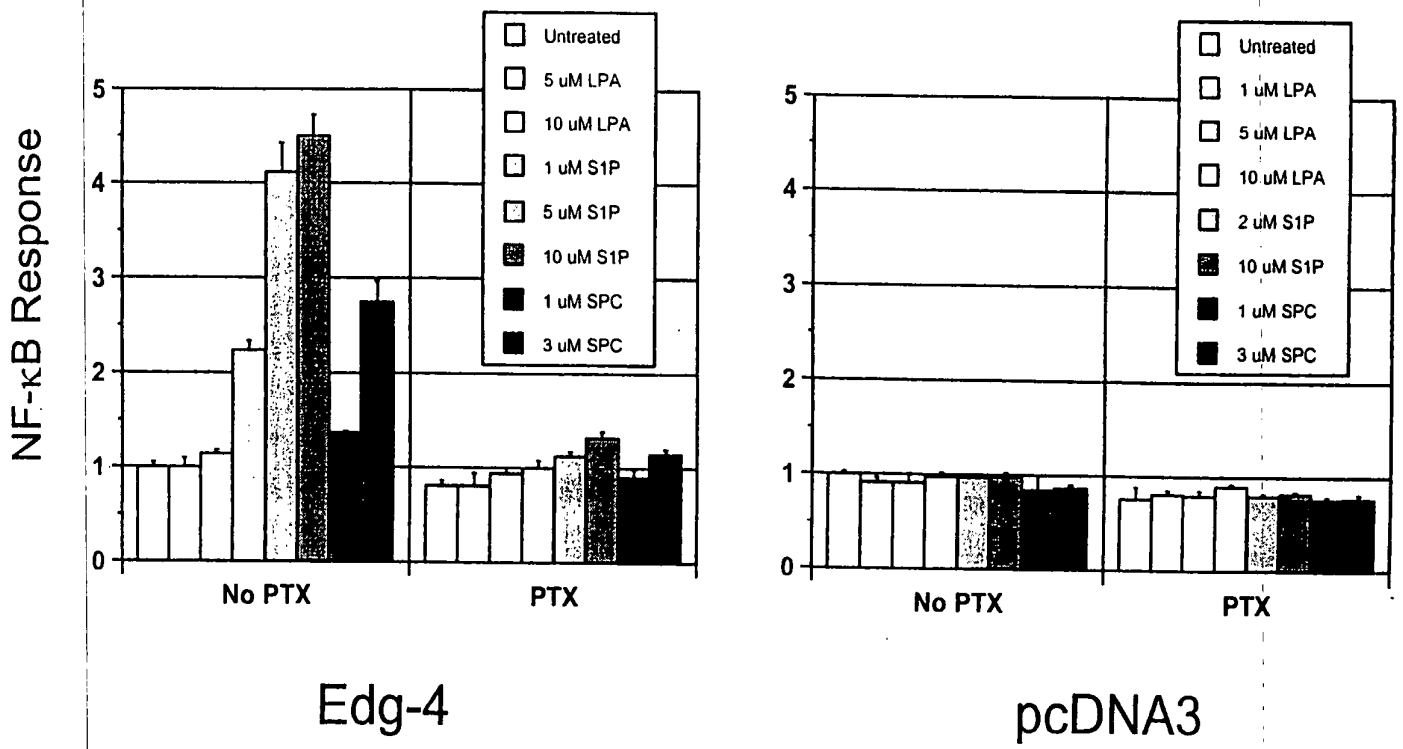

NF-κB Response
Untreated
5 uM LPA
10 uM LPA
1 uM S1P
5 uM S1P
10 uM S1P
1 uM SPC
3 uM SPC
No PTX
PTX
Edg-4
Untreated
1 uM LPA
5 uM LPA
10 uM LPA
2 uM S1P
10 uM S1P
1 uM SPC
3 uM SPC
No PTX
PTX
pcDNA3

Figure 12.

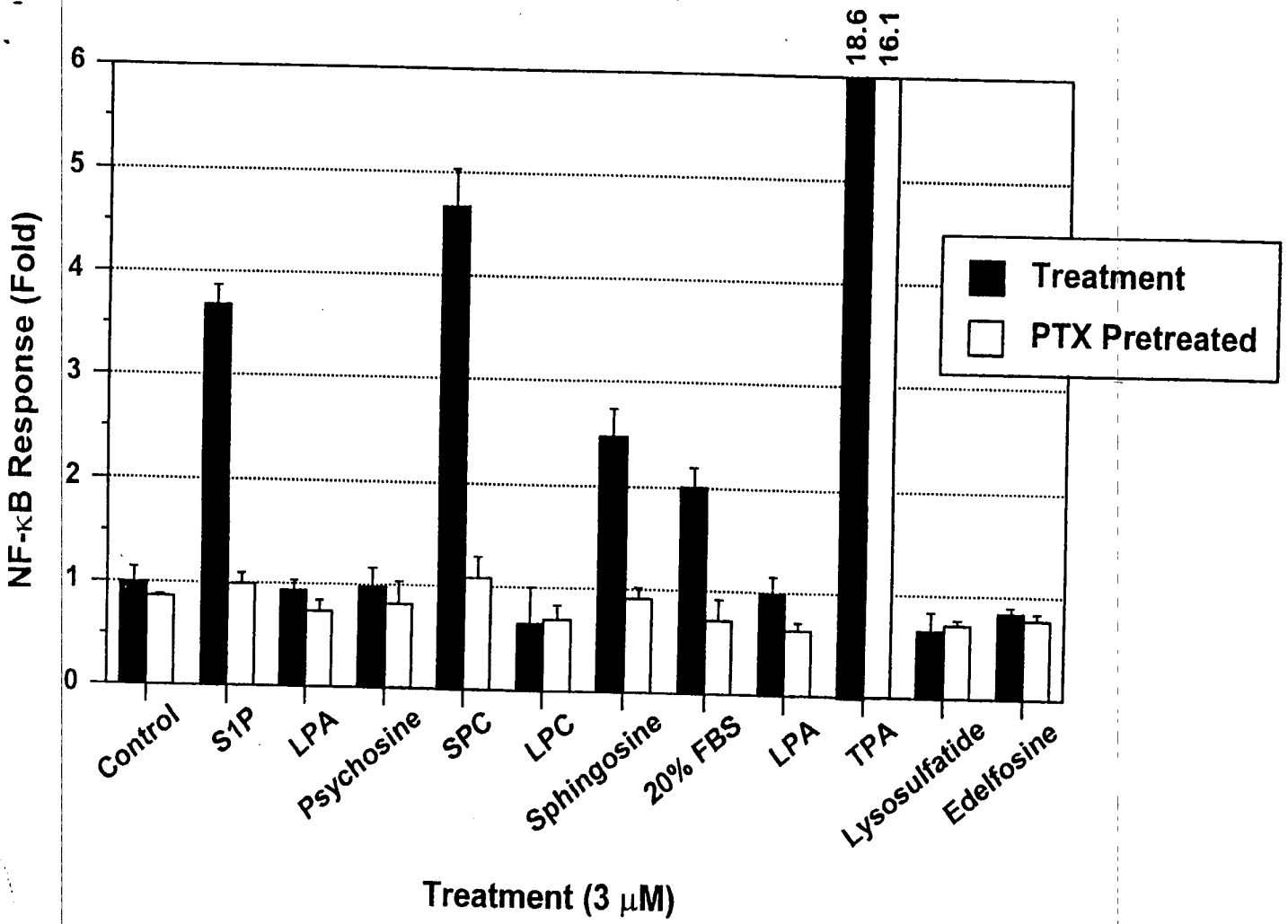

NF-κB Response (Fold)
0
1
2
3
4
5
6
18.6
16.1
Treatment
PTX Pretreated
Control
S1P
LPA
Psychosine
SPC
LPC
Sphingosine
20% FBS
LPA
TPA
Lysosulfatide
Edelfosine
Treatment (3 μM)

Figure 13.

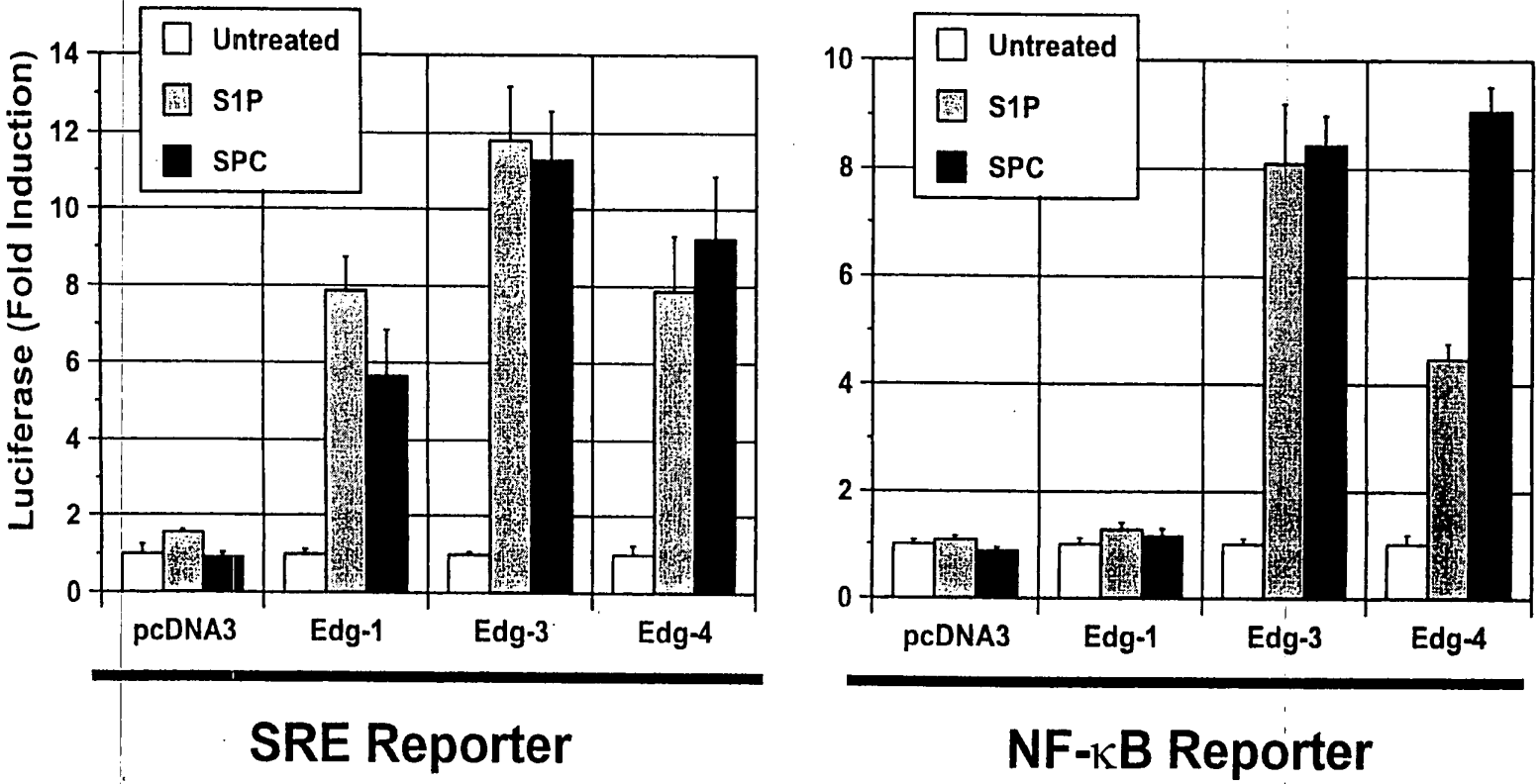
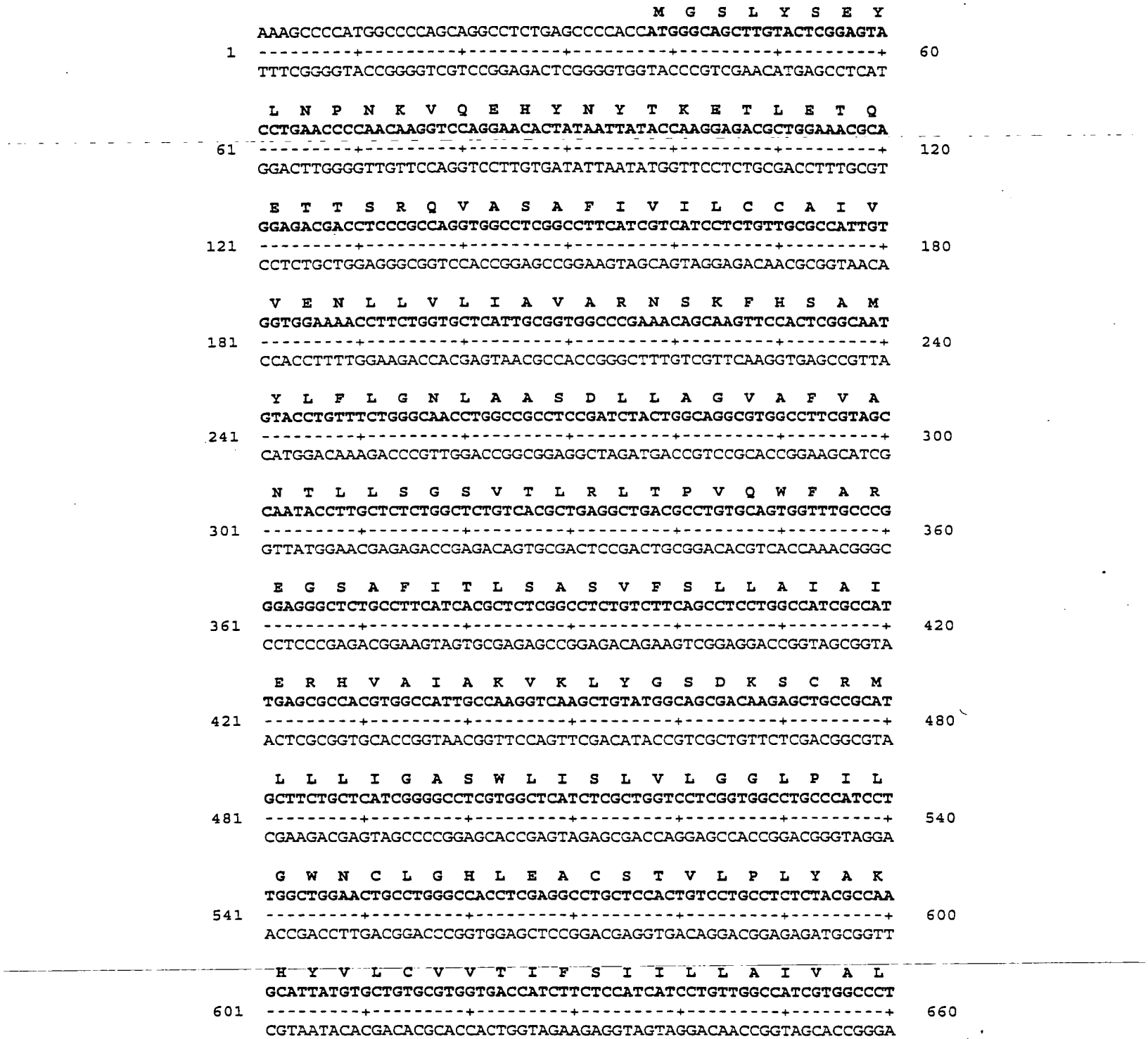

Luciferase (Fold Induction)
Untreated
S1P
SPC
0
2
4
6
8
10
12
14
pcDNA3
Edg-1
Edg-3
Edg-4
SRE Reporter
Untreated
S1P
SPC
0
2
4
6
8
10
pcDNA3
Edg-1
Edg-3
Edg-4
NF-κB Reporter

FIGURE 14

```
1                                                                   50
AA834537  --------AAA GCCCCATGGC CCCAGCAGGC CTCTGAGCCC CACCATGGGC
AA804628  --------AAA GCCCCATGGC CCCAGCAGGC CTCTGAGCCC CACCATGGGC
AA827835  AGTTCTGAAA GCCCCATGGC CCCAGCAGGC CTCTGAGCCC CACCATGGGC

          51                                                   100
AA834537  AGCTTGTACT CGGAGTACCT GAACCCCAAC AAGGTCCAGG AACACTATAA
AA804628  AGCTTGTACT CGGAGTACCT GAACCCCAAC AAGGTCCAGG AACACTATAA
AA827835  AGCTTGTACT CGGAGTACCT GAACCCCAAC AAGGTCCAGG AACACTATAA

          101                                                  150
AA834537  TTATACCAAG GAGACGCTGG AAACGCAGGA GACGACCTCC CGCCAGGTGG
AA804628  TTATACCAAG GAGACGCTGG AAACGCAGGA GACGACCTCC CGCCAGGTGG
AA827835  TTATACCAAG GAGACGCTGG AAACGCAGGA GACGACCTCC CGCCAGGTGG

          151                                                  200
AA834537  CCTCGGCATT CATCGTCATC CTCTGTTGCG CCATTGTGGT GGAAAACCTT
AA804628  CCTCGGCCTT CATCGTCATC CTCTGTTGCG CCATTGTGGT GGAAAACCTT
AA827835  GCTCGGCCTT CATCGTCATC CTCTGTTGCG CCATTGTGGT GGAAAACCTT

          201                                                  250
AA834537  CTGGTGCTCA TTGCGGTGGC CCGAAACAGC AAGTTCCACT CGGCAATGTA
AA804628  CTGGTGCTCA TTGCGGTGGC CCGAAACAGC AAGTTCCACT CGGCAATGTA
AA827835  CTGGTGCTCA TTGCGGTGGC CCGAAACAGC AAGTTCCACT CGGCAATGTA

          251                                                  300
AA834537  CCTGTTTCTG GGCAACCTGG CCGCCTCCGA TCTACTGGCA GGCGTGGCCT
AA804628  CCTGTTTCTG GGCAACCTGG CCGCCTCCGA TCTACTGGCA GGCGTGGCCT
AA827835  CCTGTTTCTG GGCAACCTGG CCGCCTCCGA TCTACTGGCA GGCGTGG.CT

          301                                                  350
AA834537  TCGTAGCCAA TACCTTGCTC TCTGGCTCTG TCACGCTGAG GCTGACGCCT
AA804628  TCGTAGCCAA TACCTTGCTC TCTGGCTCTG TCACGCTGAG GCTGACGCCT
AA827835  TCGTAGCCAA TACCTTGCTC TCTGGCTCTG TCACGCTGAG GCTGACGCCT

          351                                                  400
AA834537  GTGCAGTGGT TTGCCCGGGA CGGTCTGCCT TCATCACGCT CTCGGCCTCT
AA804628  GTGCAGTGGT TTGCCCGGGA C---------- ---------- ----------
AA827835  GTGCAGTGGT TTGCCCGGGA ---------- ---------- ----------

          401                                                  450
AA834537  GTCTTCAGCC TCCTGGCCAT CGCCATTGAG CGCCACGTGG CCATTGCAAA
AA804628  ---------- ---------- ---------- ---------- ----------
AA827835  ---------- ---------- ---------- ---------- ----------

          451
AA834537  GG
AA804628  --
AA827835  --
```

FIGURE 15 A


M G S L Y S E Y
AAAGCCCCATGGCCCCAGCAGGCCTCTGAGCCCCACCATGGGCAGCTTGTACTCGGAGTA
1 60
TTTCGGGGTACCGGGGTCGTCCGGAGACTCGGGGTGGTACCCGTCGAACATGAGCCTCAT
L N P N K V Q E H Y N Y T K E T L E T Q
CCTGAACCCCAACAAGGTCCAGGAACACTATAATTATACCAAGGAGACGCTGGAAACGCA
61 120
GGACTTGGGGTTGTTCCAGGTCCTTGTGATATTAATATGGTTCCTCTGCGACCTTTGCGT
E T T S R Q V A S A F I V I L C C A I V
GGAGACGACCTCCCGCCAGGTGGCCTCGGCCTTCATCGTCATCCTCTGTTGCGCCATTGT
121 180
CCTCTGCTGGAGGGCGGTCCACCGGAGCCGGAAGTAGCAGTAGGAGACAACGCGGTAACA
V E N L L V L I A V A R N S K F H S A M
GGTGGAAAACCTTCTGGTGCTCATTGCGGTGGCCCGAAACAGCAAGTTCCACTCGGCAAT
181 240
CCACCTTTTGGAAGACCACGAGTAACGCCACCGGGCTTTGTCGTTCAAGGTGAGCCGTTA
Y L F L G N L A A S D L L A G V A F V A
GTACCTGTTTCTGGGCAACCTGGCCGCCTCCGATCTACTGGCAGGCGTGGCCTTCGTAGC
241 300
CATGGACAAAGACCCGTTGGACCGGCGGAGGCTAGATGACCGTCCGCACCGGAAGCATCG
N T L L S G S V T L R L T P V Q W F A R
CAATACCTTGCTCTCTGGCTCTGTCACGCTGAGGCTGACGCCTGTGCAGTGGTTTGCCCG
301 360
GTTATGGAACGAGAGACCGAGACAGTGCGACTCCGACTGCGGACACGTCACCAAACGGGC
E G S A F I T L S A S V F S L L A I A I
GGAGGGCTCTGCCTTCATCACGCTCTCGGCCTCTGTCTTCAGCCTCCTGGCCATCGCCAT
361 420
CCTCCCGAGACGGAAGTAGTGCGAGAGCCGGAGACAGAAGTCGGAGGACCGGTAGCGGTA
E R H V A I A K V K L Y G S D K S C R M
TGAGCGCCACGTGGCCATTGCCAAGGTCAAGCTGTATGGCAGCGACAAGAGCTGCCGCAT
421 480
ACTCGCGGTGCACCGGTAACGGTTCCAGTTCGACATACCGTCGCTGTTCTCGACGGCGTA
L L L I G A S W L I S L V L G G L P I L
GCTTCTGCTCATCGGGGCCTCGTGGCTCATCTCGCTGGTCCTCGGTGGCCTGCCCATCCT
481 540
CGAAGACGAGTAGCCCCGGAGCACCGAGTAGAGCGACCAGGAGCCACCGGACGGGTAGGA
G W N C L G H L E A C S T V L P L Y A K
TGGCTGGAACTGCCTGGGCCACCTCGAGGCCTGCTCCACTGTCCTGCCTCTCTACGCCAA
541 600
ACCGACCTTGACGGACCCGGTGGAGCTCCGGACGAGGTGACAGGACGGAGAGATGCGGTT
H Y V L C V V T I F S I I L L A I V A L
GCATTATGTGCTGTGCGTGGTGACCATCTTCTCCATCATCCTGTTGGCCATCGTGGCCCT
601 660
CGTAATACACGACACGCACCACTGGTAGAAGAGGTAGTAGGACAACCGGTAGCACCGGGA

```
      Y  V  R  I  Y  C  V  V  R  S  S  H  A  D  M  A  A  P  Q  T
     GTACGTGCGCATCTACTGCGTGGTCCGCTCAAGCCACGCTGACATGGCCGCCCCGCAGAC
661  ---------+---------+---------+---------+---------+---------+---------+  720
     CATGCACGCGTAGATGACGCACCAGGCGAGTTCGGTGCGACTGTACCGGCGGGGCGTCTG

      L  A  L  L  K  T  V  T  I  V  L  G  V  F  I  V  C  W  L  P
     GCTAGCCCTGCTCAAGACGGTCACCATCGTGCTAGGCGTCTTTATCGTCTGCTGGCTGCC
721  ---------+---------+---------+---------+---------+---------+---------+  780
     CGATCGGGACGAGTTCTGCCAGTGGTAGCACGATCCGCAGAAATAGCAGACGACCGACGG

      A  F  S  I  L  L  L  D  Y  A  C  P  V  H  S  C  P  I  L  Y
     CGCCTTCAGCATCCTCCTTCTGGACTATGCCTGTCCCGTCCACTCCTGCCCGATCCTCTA
781  ---------+---------+---------+---------+---------+---------+---------+  840
     GCGGAAGTCGTAGGAGGAAGACCTGATACGGACAGGGCAGGTGAGGACGGGCTAGGAGAT

      K  A  H  Y  X  F  A  V  S  T  L  N  S  L  L  N  P  V  I  Y
     CAAAGCCCACTACYTTTTCGCCGTCTCCACCCTGAATTCCCTGCTCAACCCCGTCATCTA
841  ---------+---------+---------+---------+---------+---------+---------+  900
     GTTTCGGGTGATGRAAAAGCGGCAGAGGTGGGACTTAAGGGACGAGTTGGGGCAGTAGAT

      T  W  R  S  R  D  L  R  R  E  V  L  R  P  L  Q  C  W  R  P
     CACGTGGCGCAGCCGGGACCTGCGGCGGGAGGTGCTTCGGCCGCTGCAGTGCTGGCGGCC
901  ---------+---------+---------+---------+---------+---------+---------+  960
     GTGCACCGCGTCGGCCCTGGACGCCGCCCTCCACGAAGCCGGCGACGTNACGACCGCCGG

      G  V  G  V  Q  G  R  R  R  G  G  T  P  G  H  H  L  L  P  L
     GGGGGTGGGGGTGCAAGGACGGAGGCGGGGCGGGACCCCGGGCCACCACCTCCTGCCACT
961  ---------+---------+---------+---------+---------+---------+---------+ 1020
     CCCCCACCCCCACGTTCCTGCCTCCGCCCCGCCCTGGGGCCCGGTGGTGGAGGACGGTGA

      R  S  S  S  S  L  E  R  G  M  H  M  P  T  S  P  T  F  L  E
     CCGCAGCTCCAGCTCCCTGGAGAGGGGCATGCACATGCCCACGTCACCCACGTTTCTGGA
1021 ---------+---------+---------+---------+---------+---------+---------+ 1080
     GGCGTCGAGGTCGAGGGACCTCTCCCCGTACGTGTACGGGTGCAGTGGGTGAAAAGACCT

      G  N  T  V  V  *
     GGGCAACACGGTGGTCTGAGGGTGGGGGTGGACCAACAACCAGGCCAGGGCATAGGGGTT
1081 ---------+---------+---------+---------+---------+---------+---------+ 1140
     CCCGTTGTGCCACCAGACTCCCACCCCCACCTGGTTGTTGGTCCGGTCCCGTATCCCCAA

     CATGGAAAGGCCACTGGGTGACCCCAAATA
1141 ---------+---------+---------+  1170
     GTACCTTTCCGGTGACCCACTGGGGTTTAT
```

Figure 15B

cDNA sequence of clone pC3-hedg4#36 encoding functional HEDG4 receptor protein.

```
      ATGGGCAGCTTGTACTCGGAGTACCTGAACCCCAACAAGGTCCAGGAACACTATAATTAT
    1 ---------+---------+---------+---------+---------+---------+   60
      TACCCGTCGAACATGAGCCTCATGGACTTGGGGTTGTTCCAGGTCCTTGTGATATTAATA

      ACCAAGGAGACGCTGGAAACGCAGGAGACGACCTCCCGCCAGGTGGCCTCGGCCTTCATC
   61 ---------+---------+---------+---------+---------+---------+  120
      TGGTTCCTCTGCGACCTTTGCGTCCTCTGCTGGAGGGCGGTCCACCGGAGCCGGAAGTAG

      GTCATCCTCTGTTGCGCCATTGTGGTGGAAAACCTTCTGGTGCTCATTGCGGTGGCCCGA
  121 ---------+---------+---------+---------+---------+---------+  180
      CAGTAGGAGACAACGCGGTAACACCACCTTTTGGAAGACCACGAGTAACGCCACCGGGCT

      AACAGCAAGTTCCACTCGGCAATGTACCTGTTTCTGGGCAACCTGGCCGCCTCCGATCTA
  181 ---------+---------+---------+---------+---------+---------+  240
      TTGTCGTTCAAGGTGAGCCGTTACATGGACAAAGACCCGTTGGACCGGCGGAGGCTAGAT

      CTGGCAGGCGTGGCCTTCGTAGCCAATACCTTGCTCTCTGGCTCTGTCACGCTGAGGCTG
  241 ---------+---------+---------+---------+---------+---------+  300
      GACCGTCCGCACCGGAAGCATCGGTTATGGAACGAGAGACCGAGACAGTGCGACTCCGAC

      ACGCCTGTGCAGTGGTTTGCCCGGGAGGGCTCTGCCTTCATCACGCTCTCGGCCTCTGTC
  301 ---------+---------+---------+---------+---------+---------+  360
      TGCGGACACGTCACCAAACGGGCCCTCCCGAGACGGAAGTAGTGCGAGAGCCGGAGACAG

      TTCAGCCTCCTGGCCATCGCCATTGAGCGCCACGTGGCCATTGCCAAGGTCAAGCTGTAT
  361 ---------+---------+---------+---------+---------+---------+  420
      AAGTCGGAGGACCGGTAGCGGTAACTCGCGGTGCACCGGTAACGGTTCCAGTTCGACATA

      GGCAGCGACAAGAGCTGCCGCATGCTTCTGCTCATCGGGGCCTCGTGGCTCATCTCGCTG
  421 ---------+---------+---------+---------+---------+---------+  480
      CCGTCGCTGTTCTCGACGGCGTACGAAGACGAGTAGCCCCGGAGCACCGAGTAGAGCGAC

      GTCCTCGGTGGCCTGCCCATCCTTGGCTGGAACTGCCTGGGCCACCTCGAGGCCTGCTCC
  481 ---------+---------+---------+---------+---------+---------+  540
      CAGGAGCCACCGGACGGGTAGGAACCGACCTTGACGGACCCGGTGGAGCTCCGGACGAGG

      ACTGTCCTGCCTCTCTACGCCAAGCATTATGTGCTGTGCGTGGTGACCATCTTCTCCATC
  541 ---------+---------+---------+---------+---------+---------+  600
      TGACAGGACGGAGAGATGCGGTTCGTAATACACGACACGCACCACTGGTAGAAGAGGTAG

      ATCCTGTTGGCCGTCGTGGCCCTGTACGTGCGCATCTACTGCGTGGTCCGCTCAAGCCAC
  601 ---------+---------+---------+---------+---------+---------+  660
      TAGGACAACCGGCAGCACCGGGACATGCACGCGTAGATGACGCACCAGGCGAGTTCGGTG

      GCTGACATGGCCGCCCCGCAGACGCTAGCCCTGCTCAAGACGGTCACCATCGTGCTAGGC
  661 ---------+---------+---------+---------+---------+---------+  720
      CGACTGTACCGGCGGGGCGTCTGCGATCGGGACGAGTTCTGCCAGTGGTAGCACGATCCG

      GTCTTTATCGTCTGCTGGCTGCCCGCCTTCAGCATCCTCCTTCTGGACTATGCCTGTCCC
  721 ---------+---------+---------+---------+---------+---------+  780
```

```
      CAGAAATAGCAGACGACCGACGGGCGGAAGTCGTAGGAGGAAGACCTGATACGGACAGGG

      GTCCACTCCTGCCCGATCCTCTACAAAGCCCACTACCTTTTCGCCGTCTCCACCCTGAAT
781   ---------+---------+---------+---------+---------+---------+   840
      CAGGTGAGGACGGGCTAGGAGATGTTTCGGGTGATGGAAAAGCGGCAGAGGTGGGACTTA

      TCCCTGCTCAACCCCGTCATCTACACGTGGCGCAGCCGGGACCTGCGGCGGGAGGTGCTT
841   ---------+---------+---------+---------+---------+---------+   900
      AGGGACGAGTTGGGGCAGTAGATGTGCACCGCGTCGGCCCTGGACGCCGCCCTCCACGAA

      CGGCCGCTGCAGTGCTGGCGGCCGGGGGTGGGGGTGCAAGGACGGAGGCGGGGCGGGACC
901   ---------+---------+---------+---------+---------+---------+   960
      GCCGGCGACGTCACGACCGCCGGCCCCCACCCCCACGTTCCTGCCTCCGCCCCGCCCTGG

      CCGGGCCACCACCTCCTGCCACTCCGCAGCTCCAGCTCCCTGGAGAGGGGCATGCACATG
961   ---------+---------+---------+---------+---------+---------+  1020
      GGCCCGGTGGTGGAGGACGGTGAGGCGTCGAGGTCGAGGGACCTCTCCCCGTACGTGTAC

      CCCACGTCACCCACGTTTCTGGAGGGCAACACGGTGGTCTGA
1021  ---------+---------+---------+---------+--  1062
      GGGTGCAGTGGGTGCAAAGACCTCCCGTTGTGCCACCAGACT
```

FIGURE 16 A

```
  1  MGSLYSEYLN PNKVQEHYNY TKETLETQET TSRQVASAFI VILCCAIVVE

 51  NLLVLIAVAR NSKFHSAMYL FLGNLAASDL LAGVAFVANT LLSGSVTLRL

101  TPVQWFAREG SAFITLSASV FSLLAIAIER HVAIAKVKLY GSDKSCRMLL

151  LIGASWLISL VLGGLPILGW NCLGHLEACS TVLPLYAKHY VLCVVTIFSI

201  ILLAIVALYV RIYCVVRSSH ADMAAPQTLA LLKTVTIVLG VFIVCWLPAF

251  SILLLDYACP VHSCPILYKA HYXFAVSTLN SLLNPVIYTW RSRDLRREVL

301  RPLQCWRPGV GVQGRRRGGT PGHHLLPLRS SSSLERGMHM PTSPTFLEGN

351  TVV*
```

Conserved features of G-protein coupled receptors include:

N-terminal extracellular domain:	Residues 1 - 36
TM-I:	Residues 37 - 57
Intracellular loop 1:	Residues 58 - 68
TM-II:	Residues 69 - 92
Extracellular loop 1:	Residues 93 - 111
TM-III:	Residues 112 - 130
Intracellular loop 2:	Residues 131 - 149
TM-IV:	Residues 150 - 168
Extracellular loop 2:	Residues 169 - 185
TM-V:	Residues 186 - 210
Intracellular loop 3:	Residues 211 - 232
TM-VI:	Residues 233 - 254
Extracellular loop 3:	Residues 255 - 266
TM-VII:	Residues 267 - 285
C-terminal cytoplasmic domain:	Residues 286 - 353

Potential post-transcriptional modification sites:

N-glycosylation:	Residues 19
Phosphorylation:	Residues 142, 145, 219, 289,332, 345
Myristylation:	Residues 141, 318

Figure 16B

Predicted amino acid sequence of HEDG4 polypeptide encoded by pC3-hedg4#36.

```
  1  MGSLYSEYLN PNKVQEHYNY TKETLETQET TSRQVASAFI VILCCAIVVE

 51  NLLVLIAVAR NSKFHSAMYL FLGNLAASDL LAGVAFVANT LLSGSVTLRL

101  TPVQWFAREG SAFITLSASV FSLLAIAIER HVAIAKVKLY GSDKSCRMLL

151  LIGASWLISL VLGGLPILGW NCLGHLEACS TVLPLYAKHY VLCVVTIFSI

201  ILLAVVALYV RIYCVVRSSH ADMAAPQTLA LLKTVTIVLG VFIVCWLPAF

251  SILLLDYACP VHSCPILYKA HYLFAVSTLN SLLNPVIYTW RSRDLRREVL

301  RPLQCWRPGV GVQGRRRGGT PGHHLLPLRS SSSLERGMHM PTSPTFLEGN

351  TVV
```

FIGURE 17 A

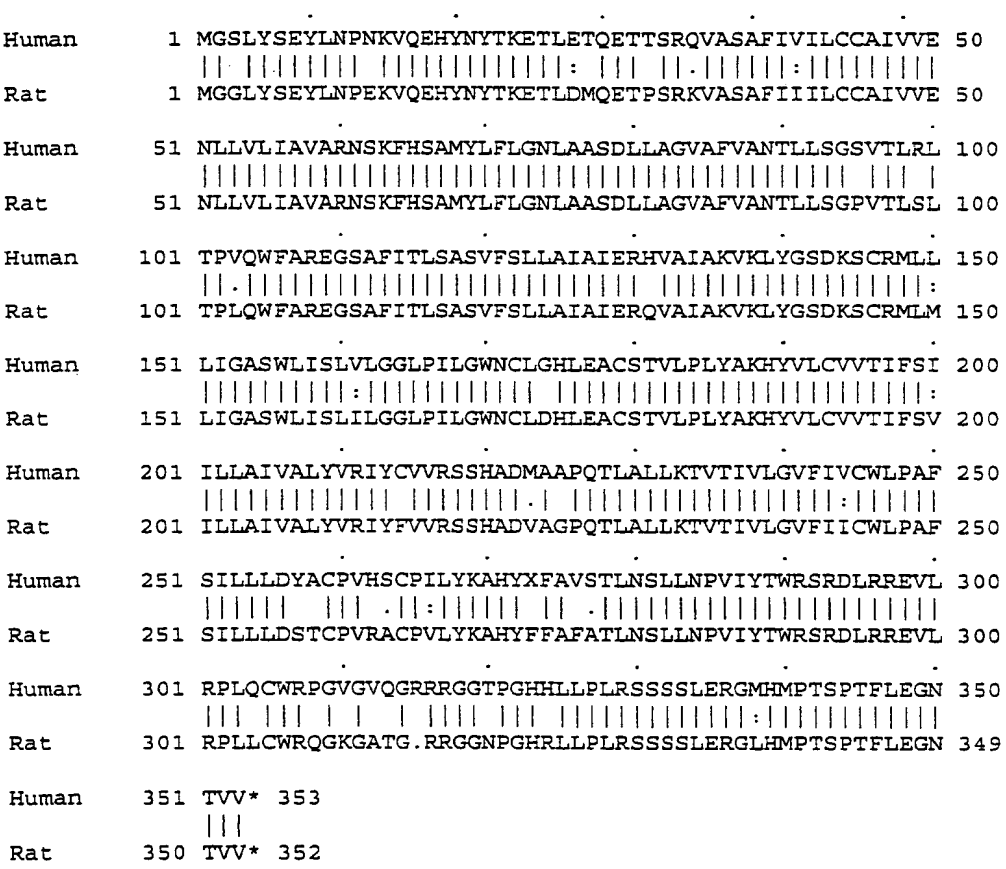

Human 1 MGSLYSEYLNPNKVQEHYNYTKETLETQETTSRQVASAFIVILCCAIVVE 50
Rat 1 MGGLYSEYLNPEKVQEHYNYTKETLDMQETPSRKVASAFIIILCCAIVVE 50
Human 51 NLLVLIAVARNSKFHSAMYLFLGNLAASDLLAGVAFVANTLLSGSVTLRL 100
Rat 51 NLLVLIAVARNSKFHSAMYLFLGNLAASDLLAGVAFVANTLLSGPVTLSL 100
Human 101 TPVQWFAREGSAFITLSASVFSLLAIAIERHVAIAKVKLYGSDKSCRMLL 150
Rat 101 TPLQWFAREGSAFITLSASVFSLLAIAIERQVAIAKVKLYGSDKSCRMLM 150
Human 151 LIGASWLISLVLGGLPILGWNCLGHLEACSTVLPLYAKHYVLCVVTIFSI 200
Rat 151 LIGASWLISLILGGLPILGWNCLDHLEACSTVLPLYAKHYVLCVVTIFSV 200
Human 201 ILLAIVALYVRIYCVVRSSHADMAAPQTLALLKTVTIVLGVFIVCWLPAF 250
Rat 201 ILLAIVALYVRIYFVVRSSHADVAGPQTLALLKTVTIVLGVFIICWLPAF 250
Human 251 SILLLDYACPVHSCPILYKAHYXFAVSTLNSLLNPVIYTWRSRDLRREVL 300
Rat 251 SILLLDSTCPVRACPVLYKAHYFFAFATLNSLLNPVIYTWRSRDLRREVL 300
Human 301 RPLQCWRPGVGVQGRRRGGTPGHHLLPLRSSSSLERGMHMPTSPTFLEGN 350
Rat 301 RPLLCWRQGKGATG.RRGGNPGHRLLPLRSSSSLERGLHMPTSPTFLEGN 349
Human 351 TVV* 353
Rat 350 TVV* 352

Figure 17B

Alignment of HEDG4 with pC3-hedg4#36 translation product and rat H218 (REDG4). Differences between pC3-hedg4#36 translation product and previously determined HEDG4 polypeptide are indicated in reverse text. Differences between rat and human edg-4 polypeptide sequences are shown in bold, shaded text.

```
            1                                                    50
   HEDG4    MGSLYSEYLN PNKVQEHYNY TKETLETQET TSRQVASAFI VILCCAIVVE
HEDG4#36    MGSLYSEYLN PNKVQEHYNY TKETLETQET TSRQVASAFI VILCCAIVVE
   REDG4    MGGLYSEYLN PEKVQEHYNY TKETLDMQET PSRKVASAFI IILCCAIVVE

            51                                                   100
   HEDG4    NLLVLIAVAR NSKFHSAMYL FLGNLAASDL LAGVAFVANT LLSGSVTLRL
HEDG4#36    NLLVLIAVAR NSKFHSAMYL FLGNLAASDL LAGVAFVANT LLSGSVTLRL
   REDG4    NLLVLIAVAR NSKFHSAMYL FLGNLAASDL LAGVAFVANT LLSGPVTLSL

            101                                                  150
   HEDG4    TPVQWFAREG SAFITLSASV FSLLAIAIER HVAIAKVKLY GSDKSCRMLL
HEDG4#36    TPVQWFAREG SAFITLSASV FSLLAIAIER HVAIAKVKLY GSDKSCRMLL
   REDG4    TPLQWFAREG SAFITLSASV FSLLAIAIER QVAIAKVKLY GSDKSCRMLM

            151                                                  200
   HEDG4    LIGASWLISL VLGGLPILGW NCLGHLEACS TVLPLYAKHY VLCVVTIFSI
HEDG4#36    LIGASWLISL VLGGLPILGW NCLGHLEACS TVLPLYAKHY VLCVVTIFSI
   REDG4    LIGASWLISL ILGGLPILGW NCLDHLEACS TVLPLYAKHY VLCVVTIFSV

            201                                                  250
   HEDG4    ILLAVVALYV RIYCVVRSSH ADMAAPQTLA LLKTVTIVLG VFIVCWLPAF
HEDG4#36    ILLAVVALYV RIYCVVRSSH ADMAAPQTLA LLKTVTIVLG VFIVCWLPAF
   REDG4    ILLAIVALYV RIYFVVRSSH ADVAGPQTLA LLKTVTIVLG VFIICWLPAF

            251                                                  300
   HEDG4    SILLLDYACP VHSCPILYKA HYXFAVSTLN SLLNPVIYTW RSRDLRREVL
HEDG4#36    SILLLDYACP VHSCPILYKA HYLFAVSTLN SLLNPVIYTW RSRDLRREVL
   REDG4    SILLLDSTCP VRACPVLYKA HYFFAFATLN SLLNPVIYTW RSRDLRREVL

            301                                                  350
   HEDG4    RPLQCWRPGV GVQGRRRGGT PGHHLLPLRS SSSLERGMHM PTSPTFLEGN
HEDG4#36    RPLQCWRPGV GVQGRRRGGT PGHHLLPLRS SSSLERGMHM PTSPTFLEGN
   REDG4    RPLLCWRQGK GATG.RRGGN PGHRLLPLRS SSSLERGLHM PTSPTFLEGN

            351
   HEDG4    TVV-
HEDG4#36    TVV-
   REDG4    TVV-
```

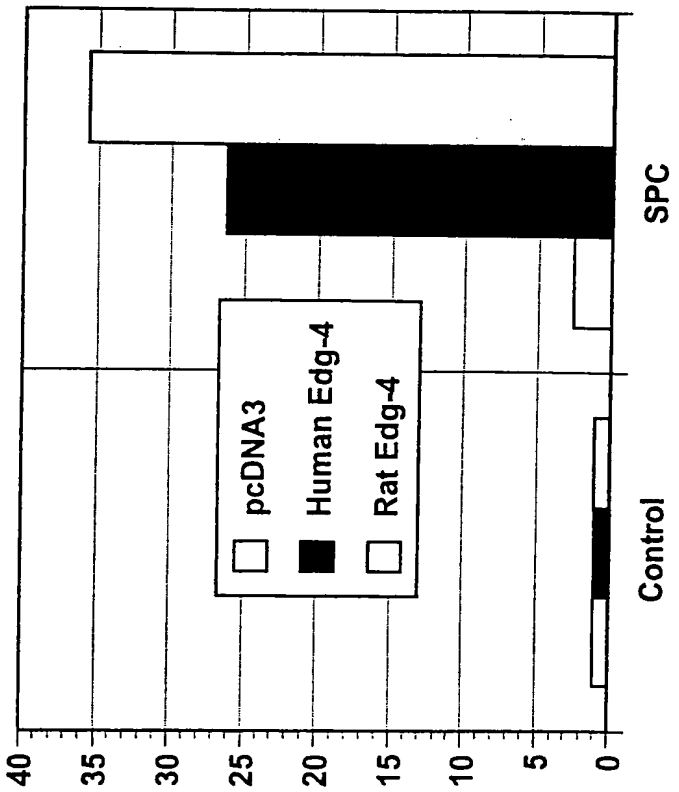


Figure 18A.

Figure 18B.

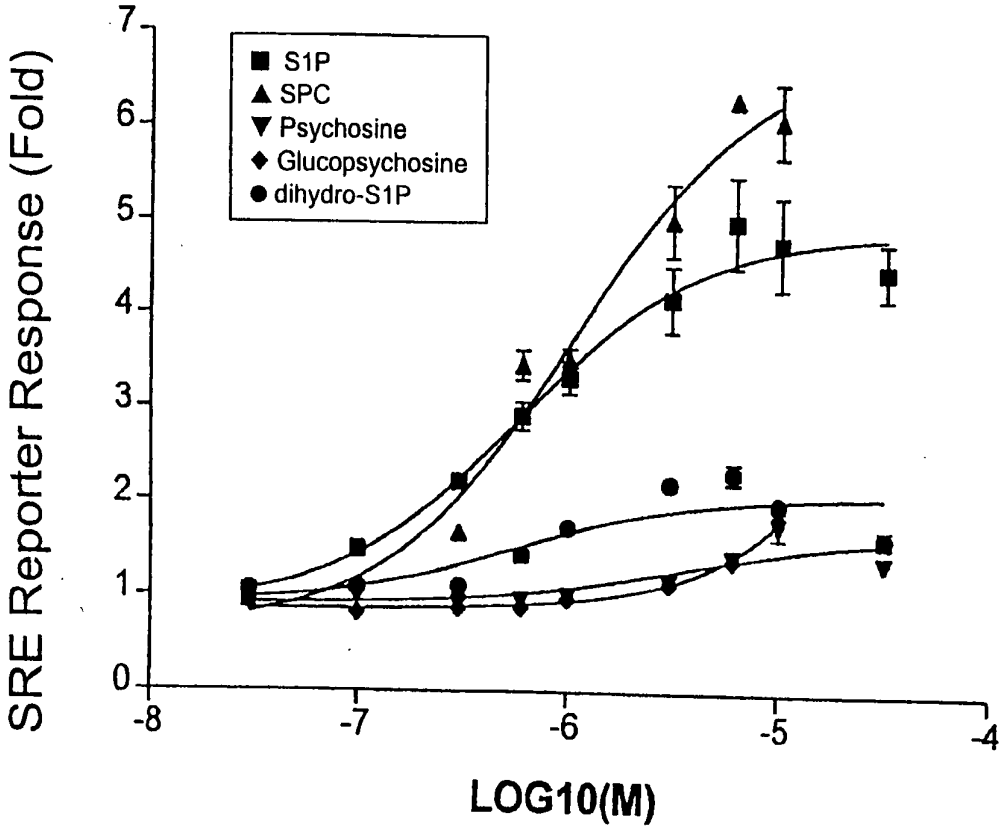

SRE Reporter Response (Fold)
LOG10(M)
S1P
SPC
Psychosine
Glucopsychosine
dihydro-S1P
0
1
2
3
4
5
6
7
-8
-7
-6
-5
-4

Figure 19.

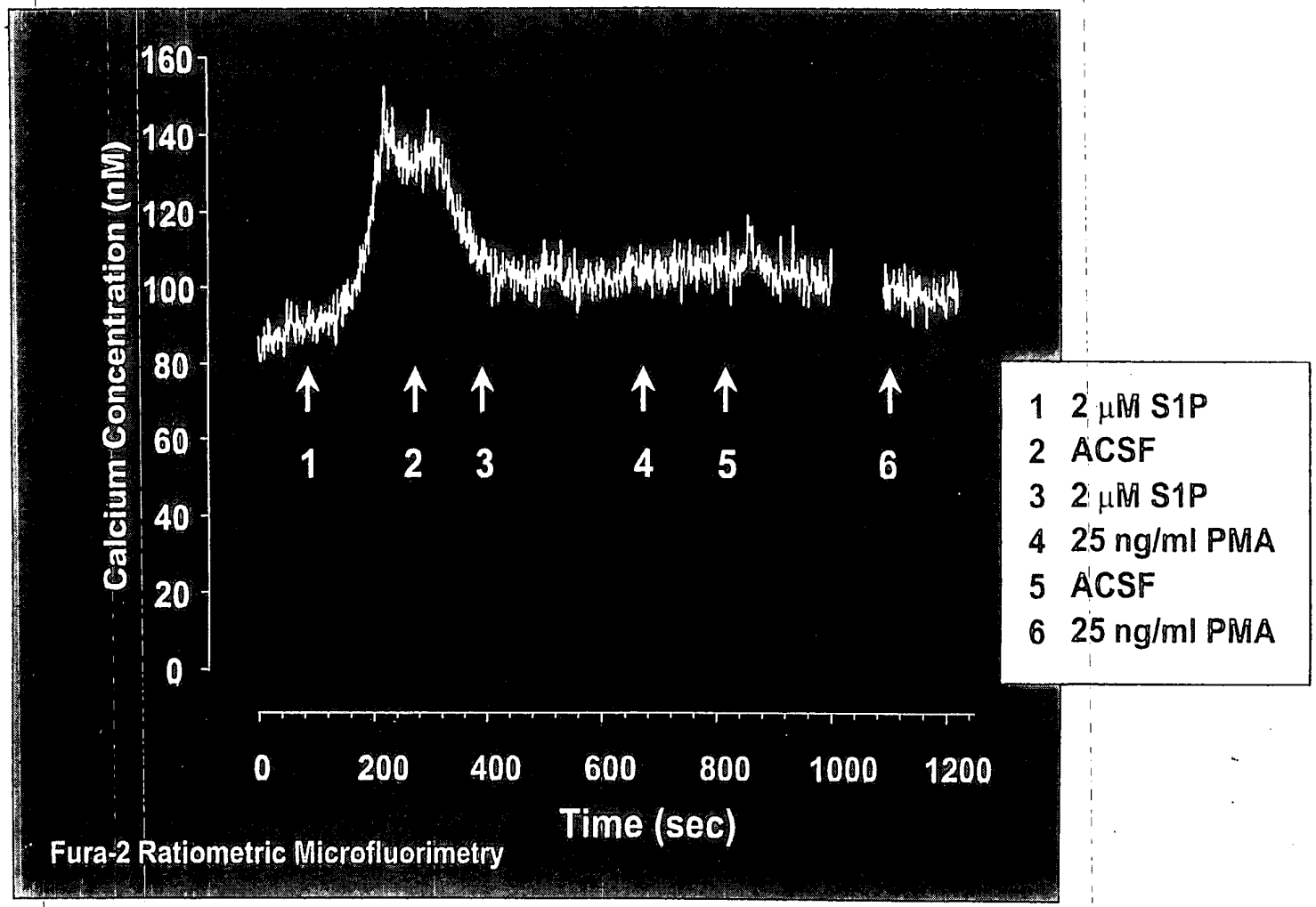

Calcium Concentration (nM)
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
0
200
400
600
800
1000
1200
Time (sec)
Fura-2 Ratiometric Microfluorimetry
1 2 μM S1P
2 ACSF
3 2 μM S1P
4 25 ng/ml PMA
5 ACSF
6 25 ng/ml PMA

Figure 20.

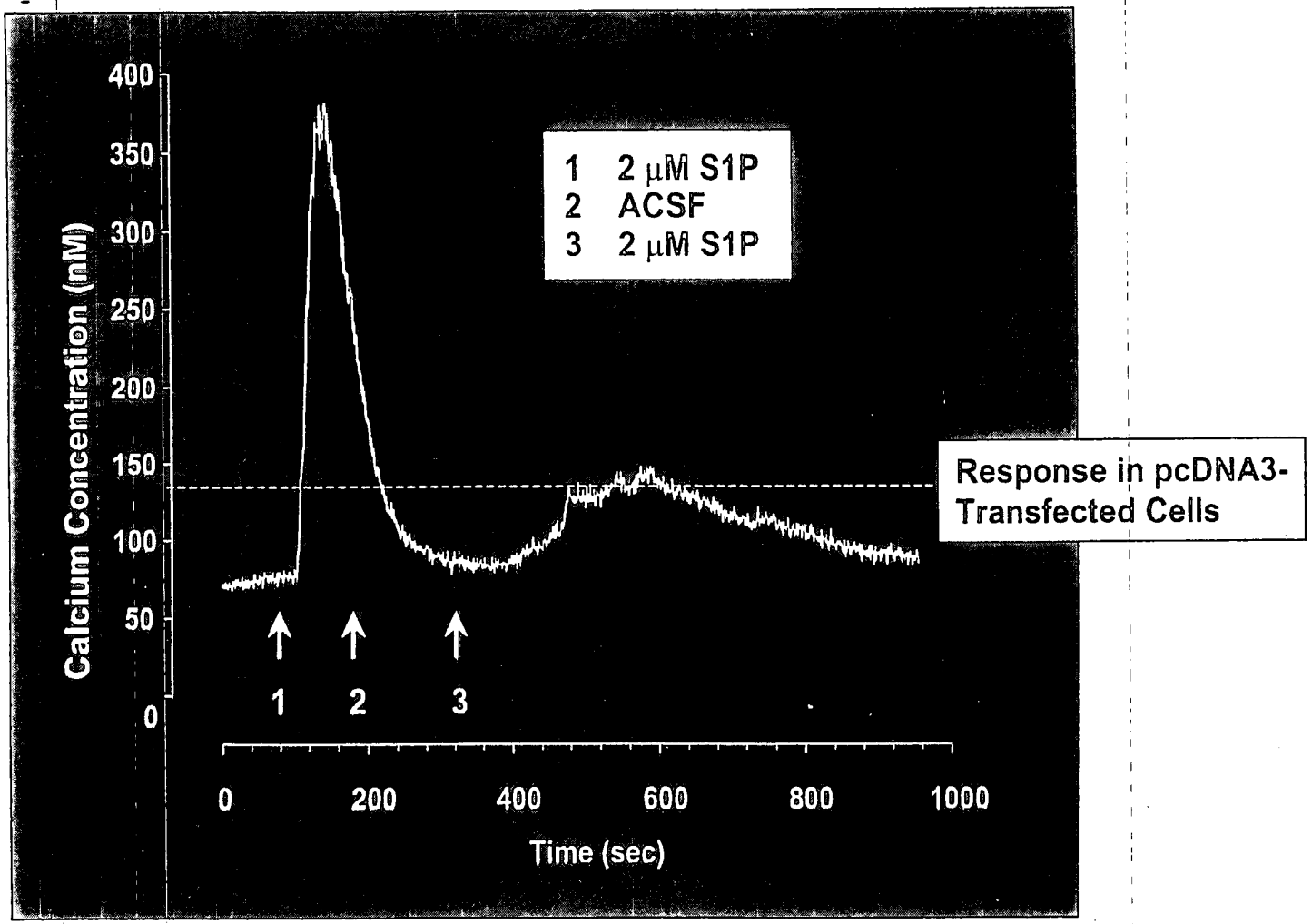

Calcium Concentration (nM)
400
350
300
250
200
150
100
50
0
1 2 μM S1P
2 ACSF
3 2 μM S1P
Response in pcDNA3-Transfected Cells
1
2
3
0
200
400
600
800
1000
Time (sec)

Figure 21. Human Edg-6 Amino Acid Sequence.

```
      MVIMGQCYYNETIGFFYNNSGKELSSHWRPKDVVVVALGLTVSVLVLLTNLLVIAAIASN
    1 ---------+---------+---------+---------+---------+---------+   60

      RRFHQPIYYLLGNLAAADLFAGVAYLFLMFHTGPRTARLSLEGWFLRQGLLDTSLTASVA
   61 ---------+---------+---------+---------+---------+---------+  120

      TLLAIAVERHRSVMAVQLHSRLPRGRVVMLIVGVWVAALGLGLLPAHSWHCLCALDRCSR
  121 ---------+---------+---------+---------+---------+---------+  180

      MAPLLSRSYLAVWALSSLLVFLLMVAVYTRIFFYVRRRVQRMAEHVSCHPRYRETTLSLV
  181 ---------+---------+---------+---------+---------+---------+  240

      KTVVIILGAFVVCWTPGQVVLLLDGLGCESCNVLAVEKYFLLLAEANSLVNAAVYSCRDA
  241 ---------+---------+---------+---------+---------+---------+  300

      EMRRTFRRLLCCACLRQSTRESVHYTSSAQGGASTRIMLPENGHPLMDSTL*
  301 ---------+---------+---------+---------+---------+--  352
```

Figure 22. Human Edg-6 Sequence

```
      ATGGTCATCATGGGCCAGTGCTACTACAACGAGACCATCGGCTTCTTCTATAACAACAGT
1     ---------+---------+---------+---------+---------+---------+    60
      TACCAGTAGTACCCGGTCACGATGATGTTGCTCTGGTAGCCGAAGAAGATATTGTTGTCA

      GGCAAAGAGCTCAGCTCCCACTGGCGGCCCAAGGATGTGGTCGTGGTGGCACTGGGGCTG
61    ---------+---------+---------+---------+---------+---------+   120
      CCGTTTCTCGAGTCGAGGGTGACCGCCGGGTTCCTACACCAGCACCACCGTGACCCCGAC

      ACCGTCAGCGTGCTGGTGCTGCTGACCAATCTGCTGGTCATAGCAGCCATCGCCTCCAAC
121   ---------+---------+---------+---------+---------+---------+   180
      TGGCAGTCGCACGACCACGACGACTGGTTAGACGACCAGTATCGTCGGTAGCGGAGGTTG

      CGCCGCTTCCACCAGCCCATCTACTACCTGCTCGGCAATCTGGCCGCGGCTGACCTCTTC
181   ---------+---------+---------+---------+---------+---------+   240
      GCGGCGAAGGTGGTCGGGTAGATGATGGACGAGCCGTTAGACCGGCGCCGACTGGAGAAG

      GCGGGCGTGGCCTACCTCTTCCTCATGTTCCACACTGGTCCCCGCACAGCCCGACTTTCA
241   ---------+---------+---------+---------+---------+---------+   300
      CGCCCGCACCGGATGGAGAAGGAGTACAAGGTGTGACCAGGGGCGTGTCGGGCTGAAAGT

      CTTGAGGGCTGGTTCCTGCGGCAGGGCTTGCTGGACACAAGCCTCACTGCGTCGGTGGCC
301   ---------+---------+---------+---------+---------+---------+   360
      GAACTCCCGACCAAGGACGCCGTCCCGAACGACCTGTGTTCGGAGTGACGCAGCCACCGG

      ACACTGCTGGCCATCGCCGTGGAGCGGCACCGCAGTGTGATGGCCGTGCAGCTGCACAGC
361   ---------+---------+---------+---------+---------+---------+   420
      TGTGACGACCGGTAGCGGCACCTCGCCGTGGCGTCACACTACCGGCACGTCGACGTGTCG

      CGCCTGCCCCGTGGCCGCGTGGTCATGCTCATTGTGGGCGTGTGGGTGGCTGCCCTGGGC
421   ---------+---------+---------+---------+---------+---------+   480
      GCGGACGGGGCACCGGCGCACCAGTACGAGTAACACCCGCACACCCACCGACGGGACCCG

      CTGGGGCTGCTGCCTGCCCACTCCTGGCACTGCCTCTGTGCCCTGGACCGCTGCTCACGC
481   ---------+---------+---------+---------+---------+---------+   540
      GACCCCGACGACGGACGGGTGAGGACCGTGACGGAGACACGGGACCTGGCGACGAGTGCG

      ATGGCACCCCTGCTCAGCCGCTCCTATTTGGCCGTCTGGGCTCTGTCGAGCCTGCTTGTC
541   ---------+---------+---------+---------+---------+---------+   600
      TACCGTGGGGACGAGTCGGCGAGGATAAACCGGCAGACCCGAGACAGCTCGGACGAACAG

      TTCCTGCTCATGGTGGCTGTGTACACCCGCATTTTCTTCTACGTGCGGCGGCGAGTGCAG
601   ---------+---------+---------+---------+---------+---------+   660
      AAGGACGAGTACCACCGACACATGTGGGCGTAAAAGAAGATGCACGCCGCCGCTCACGTC

      CGCATGGCAGAGCATGTCAGCTGCCACCCCCGCTACCGAGAGACCACGCTCAGCCTGGTC
661   ---------+---------+---------+---------+---------+---------+   720
      GCGTACCGTCTCGTACAGTCGACGGTGGGGGCGATGGCTCTCTGGTGCGAGTCGGACCAG

      AAGACTGTTGTCATCATCCTGGGGGCGTTCGTGGTCTGCTGGACACCAGGCCAGGTGGTA
721   ---------+---------+---------+---------+---------+---------+   780
      TTCTGACAACAGTAGTAGGACCCCCGCAAGCACCAGACGACCTGTGGTCCGGTCCACCAT

      CTGCTCCTGGATGGTTTAGGCTGTGAGTCCTGCAATGTCCTGGCTGTAGAAAAGTACTTC
781   ---------+---------+---------+---------+---------+---------+   840
      GACGAGGACCTACCAAATCCGACACTCAGGACGTTACAGGACCGACATCTTTTCATGAAG
```

```
      CTACTGcTGGCCGAGGCCAACTCACTGGTCAATGCTGCTGTGTACTCTTGCCGAGATGCT
841   ---------+---------+---------+---------+---------+---------+  900
      GATGACgACCGGCTCCGGTTGAGTGACCAGTTACGACGACACATGAGAACGGCTCTACGA

      GAGATGCGCCGCACCTTCCGCCGCCTTCTCTGCTGCGCGTGCCTCCGCCAGTCCACCCGC
901   ---------+---------+---------+---------+---------+---------+  960
      CTCTACGCGGCGTGGAAGGCGGCGGAAGAGACGACGCGCACGGAGGCGGTCAGGTGGGCG

      GAGTCTGTCCACTATACATCCTCTGCCCAGGGAGGTGCCAGCACTCGCATCATGCTTCCC
961   ---------+---------+---------+---------+---------+---------+ 1020
      CTCAGACAGGTGATATGTAGGAGACGGGTCCCTCCACGGTCGTGAGCGTAGTACGAAGGG

      GAGAACGGCCACCCACTGATGGACTCCACCCTTTAG
1021  ---------+---------+---------+------  1056
      CTCTTGCCGGTGGGTGACTACCTGAGGTGGGAAATC
```

Figure 23.

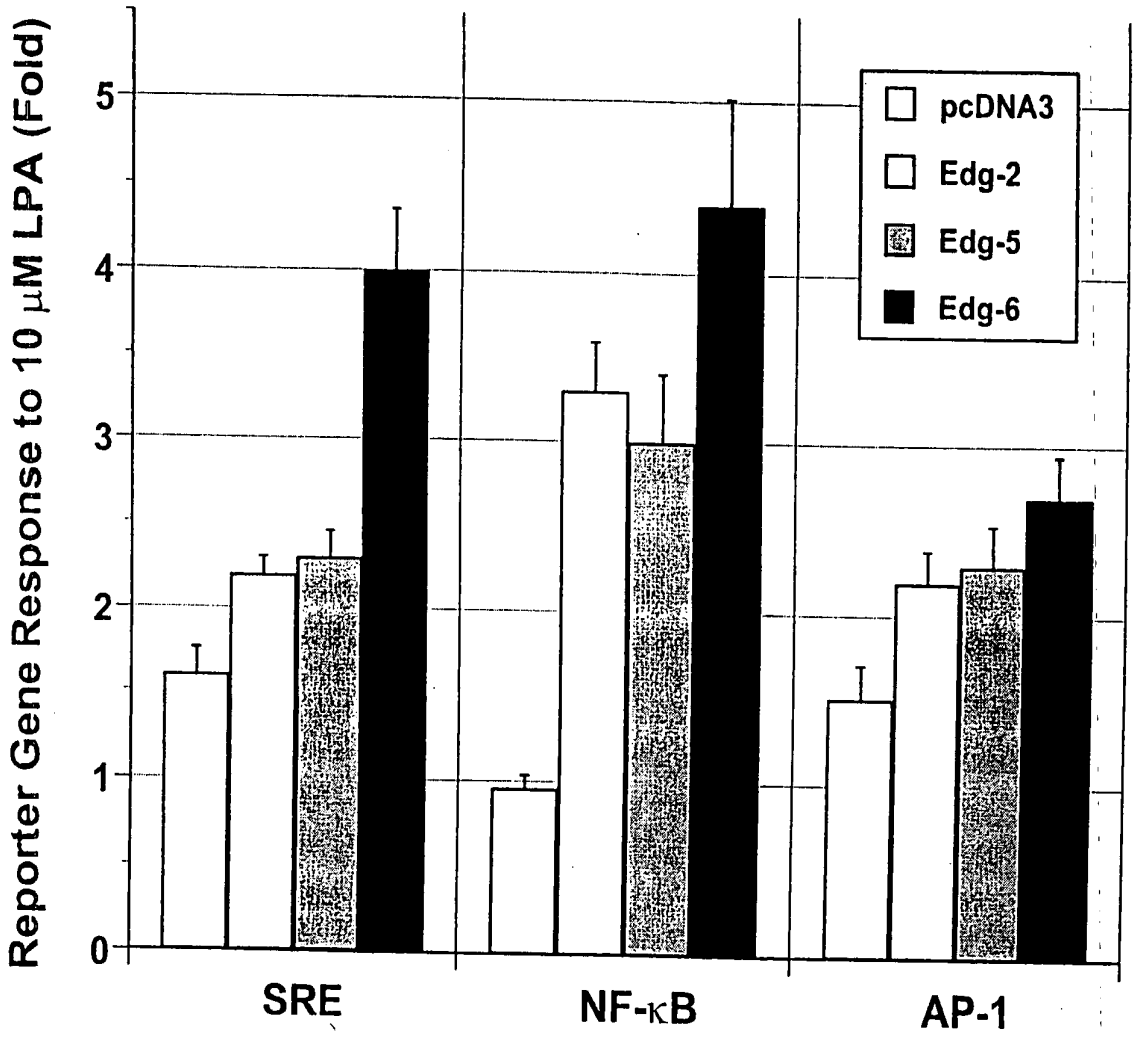

Reporter Gene Response to 10 μM LPA (Fold)
0
1
2
3
4
5
pcDNA3
Edg-2
Edg-5
Edg-6
SRE
NF-κB
AP-1

Figure 24.

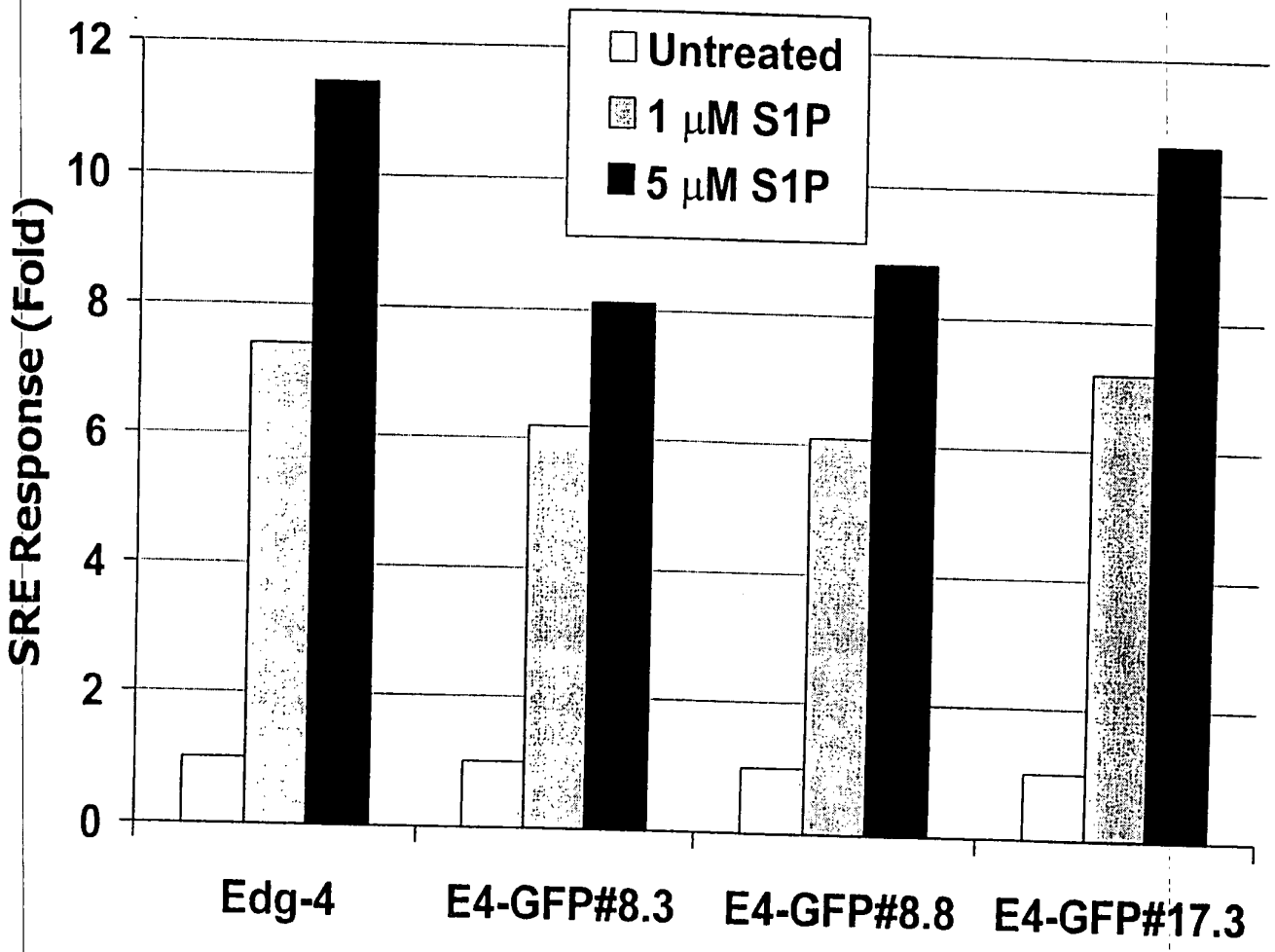
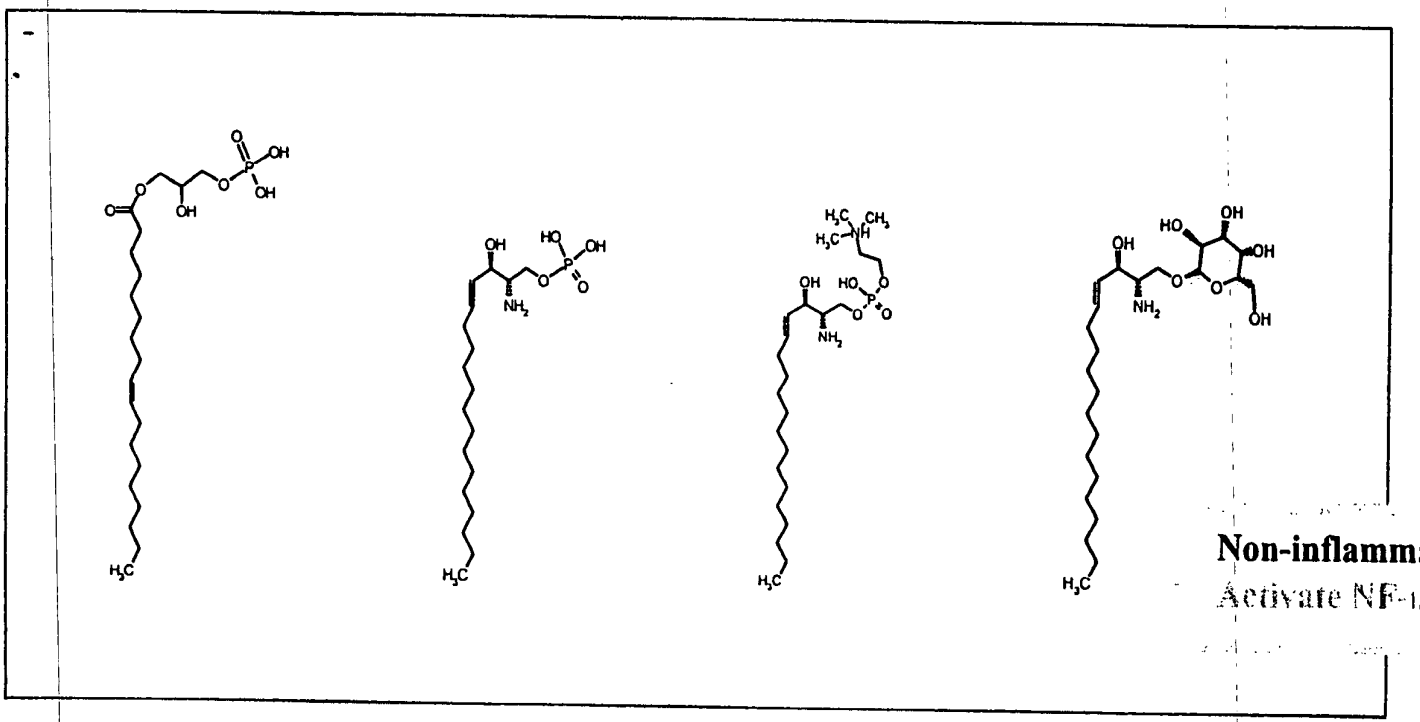

SRE Response (Fold)
12
10
8
6
4
2
0
Untreated
1 μM S1P
5 μM S1P
Edg-4
E4-GFP#8.3
E4-GFP#8.8
E4-GFP#17.3

Figure 25.



LPA	S1P	SPC	Psychosine
Edg-2	**Edg-1**	**Edg-1**	Edg-3
Edg-5	Edg-3	Edg-3	Edg-4
Edg-6	Edg-4	Edg-4	
	Edg-7	**Edg-7**	